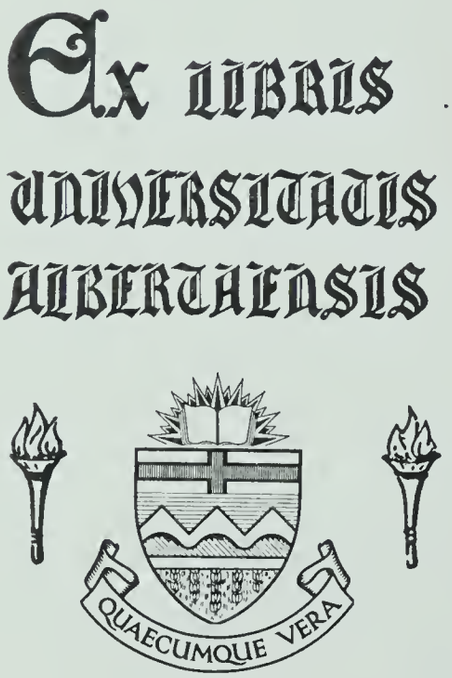

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PLASMA GLUCOSE LEVELS IN THE NORTHERN PIKE, *ESOX LUCIUS* AND THE WHITE SUCKER, *CATOSTOMUS COMMERSONII*, AND RENAL GLUCOSE REABSORPTION IN THE WHITE SUCKER

BY

© WILLIAM CHARLES MACKAY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Plasma Glucose Levels in the Northern pike, *Esox lucius*, and the White Sucker, *Catostomus commersonii* and Renal Glucose Reabsorption in the White Sucker" submitted by William Charles Mackay, in partial fulfilment for the degree of Master of Science.

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ABSTRACT

Plasma glucose levels were investigated in white suckers and northern pike which had been caught at different times of the year by both gill net and trap net. Blood samples were collected for analysis within one minute of the time that the animal was first handled. Environmental temperature, sex and various morphometric measurements were recorded in an attempt to correlate any change in plasma glucose level, which might occur, with an environmental or morphological change.

Male white suckers showed a significant seasonal change in plasma glucose level but female suckers did not. Female fish of both species showed generally higher and more variable plasma glucose levels than did male fish sampled at the same time. At certain times of the year male fish of both species showed significantly lower plasma glucose levels than did female fish. Blood samples taken from fish which had either been caught by gill net or held in the laboratory, showed higher and more variable plasma glucose levels than did fish which were sampled from the trap net.

Glucose and inulin clearance studies were carried out on white suckers in order to determine the importance of intermittent and graded glomerular filtration and to determine the effect of temperature on renal function.

Linear relationships were found to exist between inulin clearance and urine flow and between glucose transport maximum and inulin clearance in the white sucker. A linear relationship was also suggested between glucose transport maximum and the percentage of filtered water which was reabsorbed.

Urine flow in the white sucker increased as temperature increased. The Q_{10} value over the range 2 to 18°C was 2.2. Increased temperature did not significantly affect tubular water reabsorption or the glucose transport mechanism.

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INTRODUCTION

Early investigations of normal blood sugar levels and their control were carried out primarily on marine fish (Simpson, 1926; McCormick and Macleod, 1925; Gray and Hall, 1930). Attempts have been made to attach adaptive significance to the blood sugar levels found in different species of fish. Gray and Hall (1930) found a correlation between activity and blood sugar level in marine fish. Active swimmers, such as the mackerel, *Pneumatophorus colias*, and the menhaden, *Brevoortia tyrannus*, had blood sugar levels of 91 and 75 mg. per cent respectively, while the sluggish puffer, *Spheroides maculatus*, and the toadfish, *Opsanus tau*, had blood sugar levels of 23 and 15 mg. per cent respectively. However, Kiermeir (1939) found no relationship between activity and blood sugar level in the species of freshwater fish that she investigated. The sluggish carp, *Cyprinus carpio*, and the tench, *Tinca vulgaris*, had blood sugar levels of 70 and 100 mg. per cent respectively, while more active species such as the char, *Salmo fontinalis (=? Salvelinus fontinalis)*, and *Trutta iridea (=? Salmo iridea =? Salmo gairdneri)*, had average blood sugar levels of 71 mg. per cent. The highest blood sugar value in any species reported by Kiermeir (1939) was 160 mg. per cent for the eel, *Anguilla vulgaris*, when it was in freshwater. She also reported that freshwater species have more variable blood sugar levels than do marine species. Kiermeir did not quantitate these variations statistically. Pavlov (1939) reported mean values and standard deviations of the blood sugar levels in the populations she sampled. The blood sugar level of the salmonids *Salmo salar* and *Salmo trutta* was 102 ± 36 mg. per cent. The pike, *Esox lucius*, however, had an average of 71 ± 8 mg.

per cent. Thus it has not been possible to show a definite relationship between fish habitat (or activity) and average blood sugar level in freshwater fish.

Seasonal changes in the blood sugar levels of fish have not been extensively investigated. Nace *et al.* (1964) reported a seasonal change in the blood concentration of both total reducing substances and glucose in the toadfish, *Opsanus tau*. Blood glucose levels varied from 116 mg. per cent in winter to less than 30 mg. per cent in summer. This is one of the species that Gray and Hall (1930) considered to be sluggish and having a low blood sugar level. Pavlov (1939) found that blood sugar levels of the pike-perch, *Lucioperca luciop*, 15 to 20 days after spawning, were comparable to the normal value of 100 to 109 mg. per cent for so-called "normal healthy" (correct translation "normal active") fish. Kiermeir (1939) could detect no change in the blood sugar level of the pike, *Esox lucius*, held in the laboratory from April 10 to June 4.

The factors which might cause seasonal changes in blood sugar levels are not clear, nor are they the same in all species. Nace *et al.* (1964) suggested that some combination of temperature and stage of the annual sexual cycle might be responsible for the observed fluctuations in blood sugar level of the toadfish. Kiermeir (1939), however, was unable to detect any effect of temperature on the blood sugar level of the trout, *Trutta iridea (=? Salmo gairdneri)* over the range 3 to 22°C. Dean and Goodnight (1964) found contradictory results for the effects of temperature on the blood glucose levels of different species of freshwater fish.

The glomerular kidney of vertebrates is a population of nephrons

each of which consists of a glomerulus and an associated renal tubule. Urine formation takes place in two stages: (1) formation of an ultrafiltrate of the blood by the glomerulus and (2) alteration of the filtrate by reabsorption and secretion in the renal tubule. In order to determine the mechanisms by which changes in urine volume and composition are achieved, various techniques have been developed to measure rate of filtrate formation and simultaneously to quantitate the function of the renal tubules. By relating the rate of filtrate formation and the rate of tubular reabsorption or secretion, an indirect evaluation can be made of the role of glomerular and tubular processes in urine formation. A linear relationship between the rate of filtrate formation and the rate of tubular reabsorption or secretion indicates that variations in filtration rate are accomplished by varying the number of functional glomeruli. Glomerular filtration under these conditions is thought to be an "all-or-none" phenomenon and is referred to as glomerular intermittency. Glomerular intermittency has been demonstrated in the flounder, *Platichthys flesus*, (Lahlou, 1966); in the Louisiana bullfrog, *Rana catesbiana*, (Forster, 1942) and in the green frog, *Rana clamitans*, (Schmidt-Nielsen and Forster, 1954); in the freshwater turtle, *Pseudemys scripta;* and in the desert tortoise, *Gopherus agassizii*, (Dantzler, 1966). Tubular reabsorption and secretion in dogs and humans is independent of glomerular filtration rate, indicating that all glomeruli are actively filtering under normal conditions. In these instances variations in filtration rate are accomplished by varying the quantity of filtrate formed by individual nephrons; this is referred to as graded filtration. Variations in the quantity of urine produced are achieved by varying the percentage of

filtrate which is reabsorbed (Smith, 1956).

The glomerular filtration rate, GFR, can be determined by measuring inulin clearances, C_{in} (Richards, Westfall and Bott, 1934; Shannon and Smith, 1935). The relative number of tubules and hence the relative number of nephrons which are functioning can be determined by measuring the rate of reabsorption of an actively reabsorbed substance such as glucose (Forster, 1942), or by measuring the rate of secretion of an actively secreted substance such as p-aminohippuric acid, PAH, (Schmidt-Nielsen and Forster, 1954) when the reabsorptive or secretory mechanism is saturated with these substances. These rates of reabsorption and secretion exhibit characteristic maximum values which are referred to as the glucose transport maximum, Tm_G, and the PAH transport maximum, Tm_{PAH}, respectively (Pitts, 1963).

The body fluids of freshwater fish are constantly being diluted by osmotic influx of water from their environment. The net influx of water is a function of body surface permeability and the area of the body surface exposed to the environment. To maintain osmotic balance, urine production must slightly exceed the net osmotic influx of water since the water taken in with food as well as metabolic water must be excreted.

Wikgren (1953) suggested that urine flow could be used as a measure of body permeability. Temperature has been shown to directly affect urine flow in the lamprey, *Petromyzon fluviatalis* (Wikgren, 1953) and in the crucian carp, *Cyprinus carassius* (Pora and Prekup, 1960). Although they did not give the temperatures at which urine flows were measured, Haywood and Clapp (1942) reported that urine flow of the white sucker, *Catostomus commersonii*, was much lower in winter than in

spring. They observed smaller differences in the urine flow of the catfish, *Ameirus nebulosus*, between winter and spring. They suggested that seasonal changes occurred in the permeability of the body surface of these two species of freshwater fish.

Temperature has also been shown to directly affect renal transport processes. Wikgren (1953) found that ion loss in both the lamprey and the crucian carp was greater at low than at higher temperatures. Forster (1953) has shown that temperature directly affects the active secretion of PAH in the tubules of the marine longhorn sculpin, *Myoxocephalus octodecimspinosus;* the Q_{10} value was about 2.0. Hickman (1965) suggested that water reabsorption was greater in northern pike, *Esox lucius*, and white sucker, *Catostomus commersonii*, at 10°C than at 4°C.

Previous studies of blood glucose and blood sugar levels in freshwater fish have been carried out on fish held in the laboratory for various periods of time before sampling (Kiermeir, 1939; Dean and Goodnight, 1964; Nace *et al.*, 1964). Because it was thought that much of the reported variation in blood sugar level of fish might have arisen from handling and confinement in laboratory tanks, an attempt was made in this study to measure normal plasma glucose levels in the common white sucker, *Catostomus commersonii* (Lacépède) and the northern pike, *Esox lucius*, (Linnaeus) in their natural environment. Samples were collected seasonally to determine whether plasma glucose levels were affected by either the sexual cycle or temperature changes in the environment. Further objectives of the present study were: to determine the relative importance of graded glomerular activity and intermittent glomerular activity in the renal function of the white

sucker and to determine the effect of temperature on urine flow, tubular water reabsorption, and glucose transport maximum in the white sucker.

MATERIALS AND METHODS

Adult white suckers, *Catostomus commersonii*, and northern pike, *Esox lucius*, weighing between 800 and 1800 grams were collected from Lac Ste. Anne and one of its tributary streams. Lac Ste. Anne is a large, eutrophic lake, 10 to 15 m. average depth, located approximately 50 miles west of Edmonton, Alberta. Most of the fish used during summer and fall were caught in a trap net (8' x 8' x 10') which was emptied weekly. Some white suckers and northern pike were obtained by gill net in the winter of 1965-66. In late April and early May, suckers were obtained by dip net during their pre-spawning migration in the tributary of Lac Ste. Anne.

HANDLING AND SAMPLING PROCEDURES

Fish Used For Plasma Glucose Study

To minimize disturbance of other fish, individual fish were quickly removed from the lifted net and stunned by a blow on the head. A blood sample was immediately taken, within one minute of the time the fish was first handled. Damaged or diseased fish were discarded.

Blood samples of 1/2 to 1 cc. were obtained by direct puncture of the caudal circulation using a 2 1/2 inch, 18 gauge needle with a heparinized 2 1/2 cc. syringe. The blood sample was stored in a capped test tube on ice until it could be centrifuged, usually within one hour of the time of sampling. The plasma was then pipetted into a 400 µl centrifuge tube (Beckman-Spinco Co.), frozen on dry ice and stored at -15°C.

Handling of Fish Used For Renal Studies

Fish to be used for renal studies were transported from Lac Ste.

Anne to the University of Alberta in a polyethylene-lined, 135 litre drum of water aerated with oxygen. In summer, the transport water was cooled with blocks of dechlorinated ice. This procedure produced cold narcosis in the fish, decreasing damage and mortality during transport. Fish were held at the laboratory in 720 litre tanks containing dechlorinated tap water which was recirculated through sand filters. Temperatures were held at desired levels by temperature controlled ($\pm$0.5°C) refrigeration units. Holding temperatures were 4°C in winter and spring and 10°C in summer and fall. Fish were held in the laboratory for at least three days but not more than two weeks before any experiments were begun. The fish were not fed.

Prior to cannulation and catheterization, fish were anaesthetized with tricaine methanesulphonate (MS 222, Sandoz Co., Basle) dissolved in dechlorinated tap water (0.20 g./l.). Operations were carried out in a plexiglass operating box which allowed the head of the fish to be covered with water while the tail region was out of the water (Fig. 1). Urinary catheters were fabricated from lengths of PE 160 tubing (Intramedic, Clay-Adams Inc.) by perforating the wall of the tubing at one end. To prevent leakage of urine after the catheter had been inserted into the bladder, a suture was placed through the dorsal side of the rectum and around the urinary duct posterior to the bladder. To prevent the catheter from being pulled out of the bladder, the catheter was tied to the anal fin (Fig. 2).

To obtain blood samples while infusing glucose into the vascular system, a double lumen cannula (Fig. 3) was placed in the caudal circulation. Both the cannula and the method used to place it in the caudal circulation were developed during 1965 at the University of

Figure 1. White sucker in the leucite container used to hold anaesthetized fish during operative procedures. The head region was covered with water while the tail region, where operative procedures were performed, was out of the water.

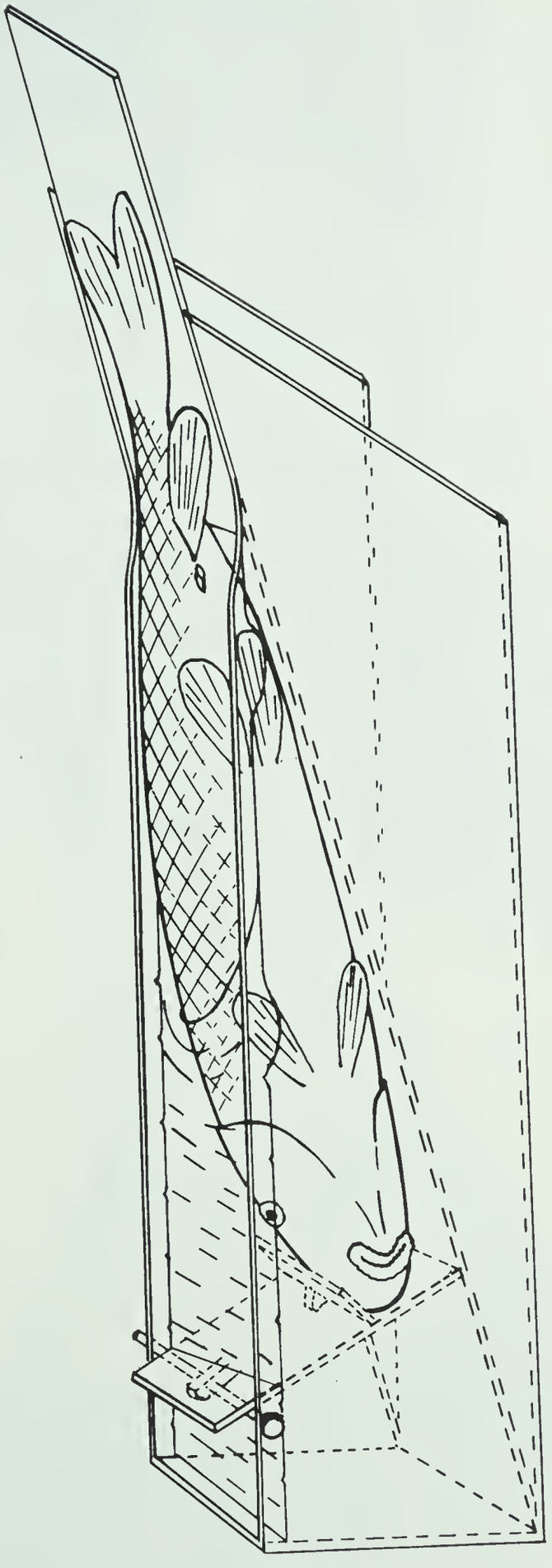


Figure 2. Caudal region of a white sucker showing the position of the cannula in the caudal vein and the catheter in the urinary bladder. Since the cannula was inserted from the ventral aspect of the caudal peduncle, it could enter either the caudal artery or the caudal vein.

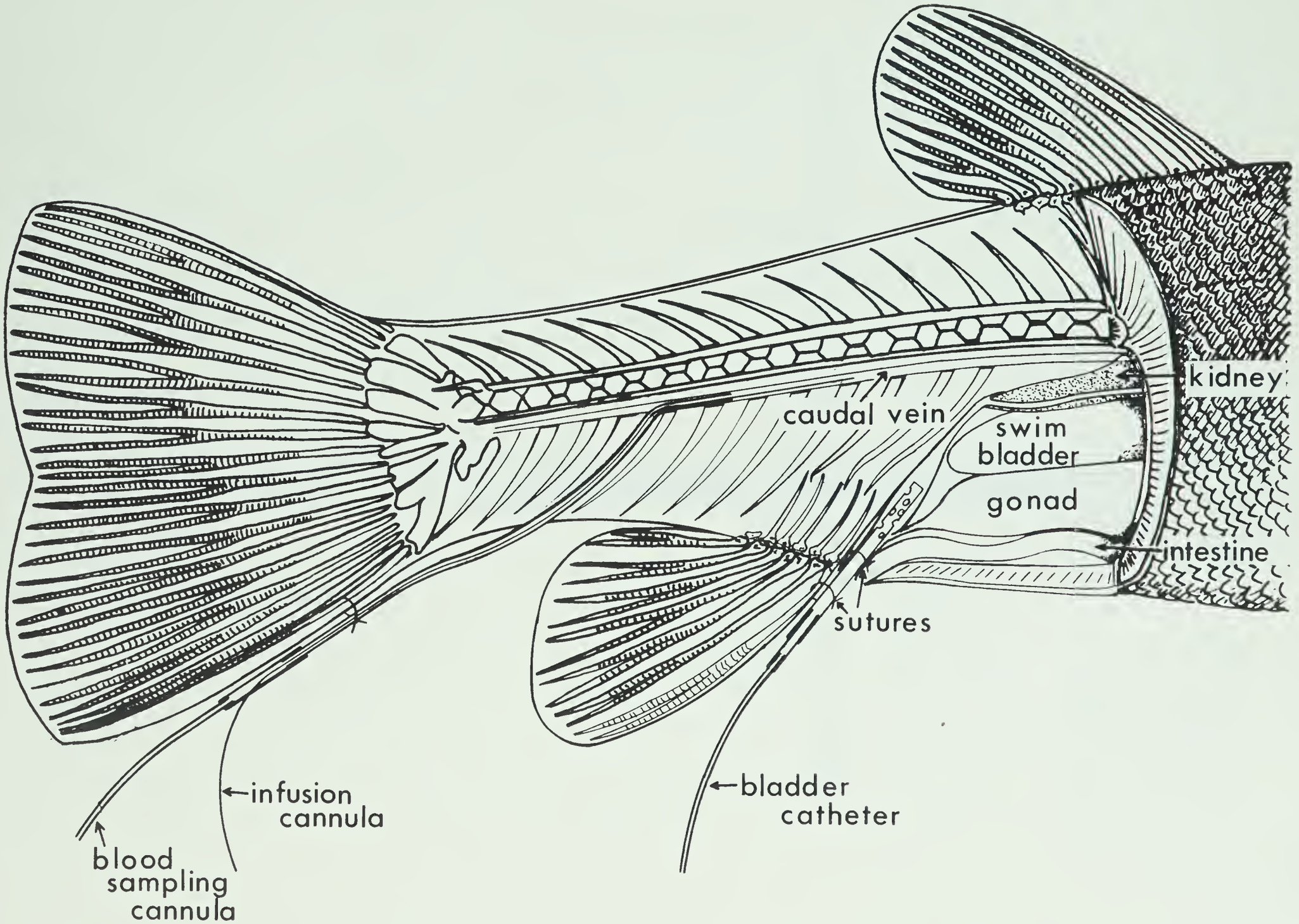
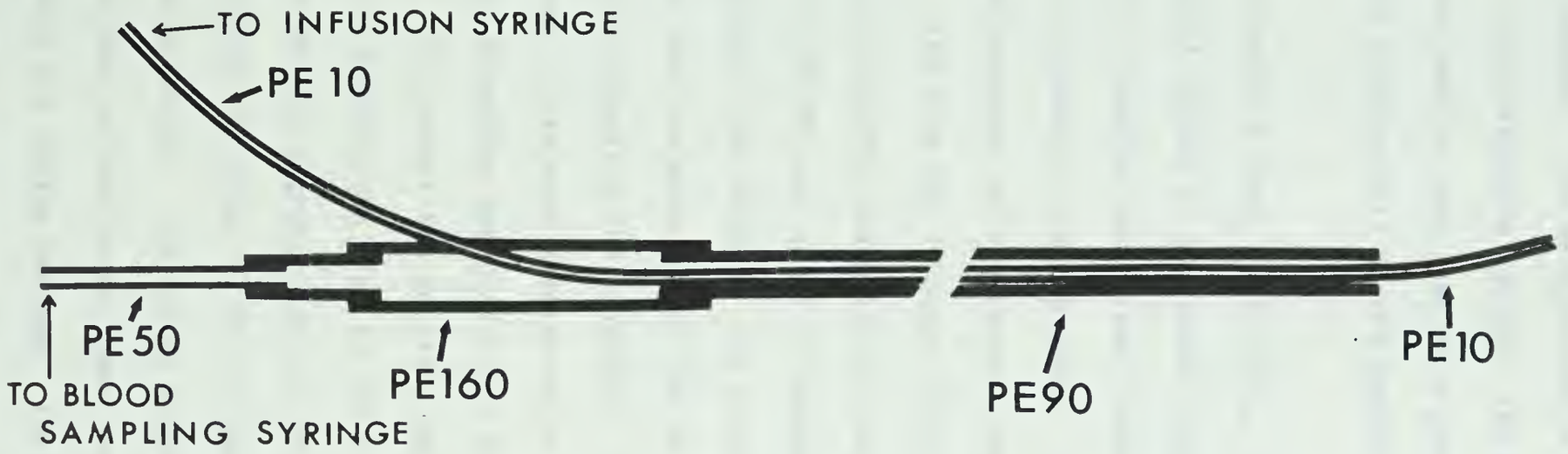

kidney
caudal vein
swim bladder
gonad
intestine
sutures
infusion cannula
blood sampling cannula
bladder catheter

Figure 3. Detail of the double lumen cannula which was inserted into the caudal circulation of the fish.

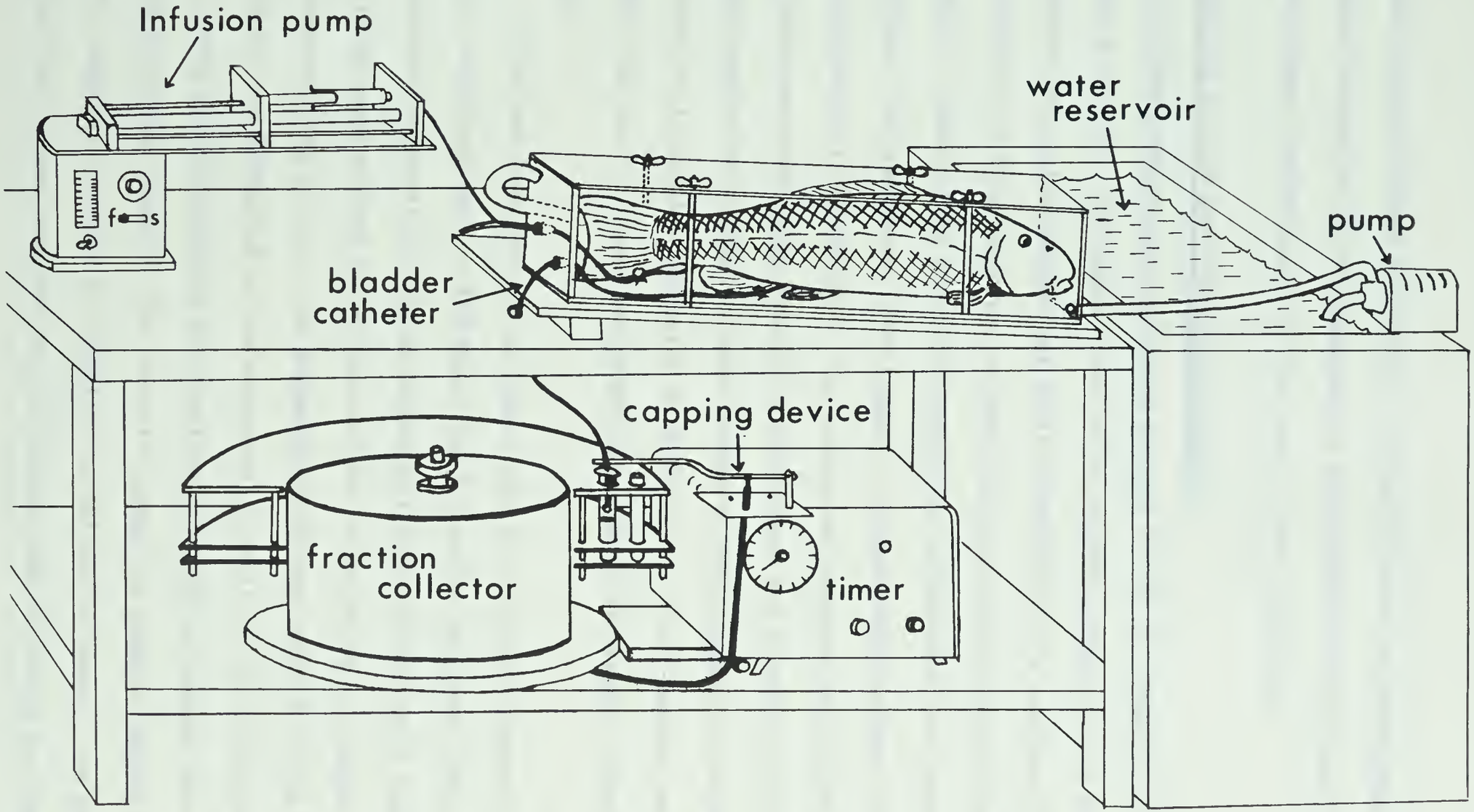

TO INFUSION SYRINGE
PE 10
PE 50
TO BLOOD
SAMPLING SYRINGE
PE160
PE90
PE10

Alberta by Mr. B. R. Hammond. The double cannula was constructed using four different sizes of polyethylene tubing. The sizes of tubing used were chosen so that the smaller tube used in a joint fit tightly inside the larger tube and the outside wall of the smaller tube held the joint together.

A heparinized, three-inch long, thin-walled, 18 gauge needle was inserted from the ventral region of the caudal peduncle into the caudal circulation. A piece of flexible wire (steel wound mandolin string) was then inserted through the needle into the vessel. The needle was removed, and the PE 90 component of the cannula was advanced over the wire into the vessel. The wire was removed, leaving the PE 90 tube in the vessel. The cannula was then assembled by pushing the PE 10 tube through the lumen of the PE 90 tube until it extended two inches past the end of the PE 90 tube in the vessel. The outside end of the PE 90 tubing was push-fitted into the open end of the PE 160 tubing at the bilumen joint (Fig. 3) to complete the assembly. The lumen of both the PE 10 and PE 90 tube was filled with a solution of 250 units heparin in saline. This latter procedure was done after every blood sampling or period of infusion. To prevent the double cannula from slipping out of the blood vessel, the cannula was tied to the caudal fin (Fig. 2).

Following cannulation and catheterization, the fish was placed in a water-tight plexiglass box (Fig. 4) for at least 24 hours prior to any experimental manipulation. The inside dimensions of the box were approximately the same as the length, depth and width of a sucker. Fish of up to 2 kg. would fit inside the box. The box received a continuous flow of aerated, dechlorinated tap water at a controlled temperature. Both catheter and cannula were led out of the box through

Figure 4. The experimental apparatus used to hold white suckers during renal studies. The leucite box in which the fish was held received water from the reservoir. Infusion was by a modified Palmer kymograph, and urine was collected by the fraction collector located immediately below the leucite box.


Infusion pump
water reservoir
pump
bladder catheter
capping device
fraction collector
timer

water-tight fittings at the rear. After 24 hours, a blood sample was taken through the PE 90 component of the cannula and was used to assay the plasma glucose level of fish held in the laboratory.

Sampling of Fish Used for Renal Studies

Blood samples were collected through the PE 90 component of the double cannula. The heparinized saline solution which filled the cannula between collection periods was first withdrawn with a syringe. This was followed by 0.25 to 0.50 cc. of blood to clear the cannula of residual heparin. Then a 0.25 cc. blood sample was collected with a separate syringe. The sample was centrifuged in a Beckman-Spinco model 152 microfuge for three minutes. The plasma was pipetted into a 400 µℓ polyethylene centrifuge tybe which was capped and stored at -15°C. Blood samples were taken at the midpoint of the one-hour urine sample collection intervals. Normally no more than 15 blood samples were taken from one fish over the sampling period which usually lasted two days.

Urine was continuously collected from the urinary catheter into tared test tubes using a LKB model 240 B fraction collector (Fig. 4). To minimize evaporative losses during collection of the urine sample, the tubes were covered during the collection period by a solenoid operated capping device. The solenoid, which was located in the fraction collector was connected to the electrical circuit of the timing device. The timing device signalled the fraction collector to change the urine collection tubes hourly. The solenoid caused the capping device to be lifted immediately prior to changing the collection tubes. The capping device then dropped onto the new collection tube following the change.

This insured minimal evaporative loss during the collection period. Following collection, the tubes were weighed, sealed with parafilm, frozen and stored at -15°C.

ANALYTICAL PROCEDURES

Measurement of Glomerular Filtration Rate

Glomerular filtration rates were measured by the inulin clearance method described by Richards, Westfall and Bott (1934) and by Shannon and Smith (1935). The use of inulin clearance as a valid measure of glomerular filtration rate has been established in *Necturus* (Tanner and Klose, 1966). In view of the close similarity between amphibian and fish kidneys, the assumption can be made that inulin clearance is also a valid measure of glomerular filtration rate in fish. Usually, ten microcuries of carboxyl-^{14}C inulin (Volk Radiochemical Co.) was injected into the circulation through the PE 10 component of the double cannula at least 12 hours prior to the first measurement of inulin clearance. Since inulin clearance in the white sucker is independent of plasma inulin concentration (Hickman, 1965) a single injection of inulin was used rather than constant infusion. In other experiments, tritium-labelled inulin was used to assay inulin clearances in fish which were infused with ^{14}C-labelled glucose. Twenty microcuries of tritium-labelled inulin was given by single injection in the same manner as the ^{14}C-labelled inulin.

The rate of inulin clearance, C_{in}, (ml./hr.) was calculated according to the formula:

$$C_{in} = \frac{U_{in} V}{P_{in}}$$

where: U_{in} equals the urine inulin concentration (cpm/50 µℓ), V equals

the rate of urine flow (ml./hr.) and P_{in} equals the plasma inulin concentration (cpm/50 µl) (Smith, 1956).

Measurement of Glucose Transport Maximum (Tm_G)

In order to saturate the renal glucose reabsorptive mechanism in the fish, a 5% (W/V) glucose solution was infused through the PE 10 component of the double cannula. The glucose solution was infused for 12 hours preceeding blood or urine collection and during the period of sample collection at a rate of 0.68 cc./hr. To determine whether the fish exhibited glucosuria, the urine was qualitatively checked for the presence of glucose, before and during the sampling periods, using Clinistix Reagent Strips (Ames Co. of Canada Ltd., Toronto). This was done by spotting a drop of urine onto the strip and observing a color change. Following enzymatic determination of the glucose concentration in blood and urine samples, and following the determination of inulin clearance, the Tm_G was calculated according to the formula:

$$Tm_G = C_{in} P_G - U_G V$$

where: U_G and P_G equal the concentration of glucose in the urine and plasma respectively (mg.%), V equals the rate of urine flow (ml./hr.) and C_{in} is the inulin clearance (ml./hr.) (Pitts, 1964).

Glucose Assay

Glucose levels in plasma and urine samples were determined enzymatically (Keston, 1956; Teller, 1956) using Glucostat reagent (Worthington Biochemical Corp.). Calibrated glass micro pipettes (Microchemical Specialties, Berkeley) were used for all volume measurements. Twenty µl plasma samples were taken from fish used for

normal plasma glucose determinations. These samples were precipitated with 20 µℓ of 1N HCl followed by 20 µℓ of 1N NaOH, centrifuged, and two 5 µℓ samples of the supernatant fluid were taken for glucose analysis.

Ten µℓ plasma samples from fish used in renal glucose reabsorption studies were precipitated with 20 µℓ of HCl followed by 20 µℓ of 1N NaOH. Following centrifugation, two 5 µℓ samples of the supernatant fluid were analysed in the same way as those from fish used in normal plasma glucose studies.

Duplicate 5 µℓ samples of urine were assayed for glucose by the same procedure as that used for precipitated plasma samples. If the urine contained more than 125 mg.% glucose, a 20 µℓ sample was diluted with 20 µℓ of 1N NaOH and 20 µℓ of 1N HCl. Duplicate 5 µℓ samples of the diluted urine were then measured by the method used for plasma supernatants.

Glucose standards from 25 to 100 mg.% were prepared daily, through dilution, from a 1000 mg.% stock solution. The stock solution of β, D-glucose (reagent grade, British Drug House) was made up weekly in distilled water and then stored at 4°C. Glucostat reagent was prepared fresh daily by the method outlined in the Beckman-Spinco Ultra Micro Analytical System. Forty 400 µℓ polyethylene test tubes, each containing 250 µℓ samples of Glucostat reagent were warmed to 37°C. Duplicate samples of each of 15 different plasma supernatants or diluted urine samples and five different glucose standard solutions were analysed at one time. Five µℓ samples of the precipitated plasma supernatants or one of the standard solutions was added to the prewarmed Glucostat and the mixture was incubated for 30 minutes at 37°C. The reaction in each of the 400 µℓ polyethylene tubes was

stopped after 30 minutes (Saifer and Gerstenfeld, 1958) by the addition of 10 µℓ of 5N HCl. A reagent blank was prepared by adding 100 µℓ of distilled water to 5 ml. of prewarmed Glucostat reagent. After 30 minutes of incubation at 37°C, 200 µℓ of 5N HCl was added to the blank. Following the addition of acid, the color was allowed to stabilize for at least ten minutes before the optical density was determined with a Beckman-Spinco Model 151 Spectro-colorimeter at a wavelength of 410 mµ (nanometres). The reagent blank was used to adjust the zero optical density before reading each new set of duplicates. The microcuvette was rinsed with distilled water and dried with methanol between readings or samples and blank and between blank and samples, but not between duplicates of the same sample. After measuring the optical densities of duplicates of five samples, the cuvette was washed by drawing the following sequence of solutions through it: distilled water, soap solution (5% FL-70, Fisher Scientific), distilled water, acid alcohol (10% con. HCl in 98% ethanol), distilled water, ethanol and air.

A standard curve relating optical density to concentration of glucose, over the range 0 to 100 mg.% glucose, was constructed using the optical densities obtained from the duplicate analyses of each of the five standards. Glucose concentrations of plasma and urine samples were calculated from the standard curve, taking into account dilution factors. The glucose concentrations of duplicate blood or urine samples were averaged.

Isotope Counting Procedures

Levels of radioactivity in plasma and urine samples containing ^{14}C labelled inulin were determined using a Nuclear-Chicago gas

flow counter. ^{14}C activity in plasma samples was determined as follows: 50 µl of plasma was plated out onto a disposable aluminum planchet containing a disc of microscope lens tissue. The tissue soaked up the plasma so that it spread evenly over the bottom of the planchet, thus providing a uniform geometry for counting. Fifty µl of non-radioactive urine, obtained from a fish which had not received any radioactive isotope, was then spread over the same planchet. When the activity of a urine sample was to be determined, 50 µl of the urine was plated out in a planchet containing a tissue disc, followed by 50 µl of non-radioactive plasma obtained from a fish which had not been given any radioactive isotope. One hundred µl of fluid was sufficient to ensure that the tissue disc was wetted thoroughly so that the inulin would be evenly distributed in it. The non-radioactive samples were used to maintain constant quenching in both plasma and urine samples. The planchets were dried under heat lamps; then the time required for 10,000 counts was determined in the gas flow counter.

Plasma and urine samples which contained both ^{14}C-labelled glucose and tritium-labelled inulin were counted on a Nuclear-Chicago Mark II liquid scintillation counter operated to differentiate between the two beta-emitting isotopes. If the activity of a plasma sample was to be determined, 50 µl of the plasma was made miscible with toluene by the addition of 200 µl of NCS reagent (Nuclear-Chicago) to the plasma in low potassium scintillation vials (Nuclear-Chicago). No non-radioactive urine sample was added due to the quenching effect of water. When urine activity was to be determined, a 50 µl sample of urine was made miscible with toluene in the same way as the plasma sample. Following solubilization of the sample, 15 µl of scintillation

fluid was added to each scintillation vial. The scintillation fluid used consisted of 0.3 g. of PPO (2.5-diphenyloxazole, scintillation grade, Nuclear-Chicago) and 5.0 g. POPOP [2-p-phenylenebis (5-phenyloxazole), scintillation grade, Nuclear-Chicago] per litre of toluene (reagent grade, Fisher Scientific). All samples were counted twice for ten minutes with the counter at the following settings: tritium channel, attenuator A 83, upper discriminator 1.1 volts, lower discriminator 0.5 volts; ^{14}C channel, attenuator D 912, upper discriminator 9.9 volts, lower discriminator 0.9 volts. The extent of quenching was determined for each sample by using the external standard method (Higahimura *et al.*, 1962). The computer was set to report the ratio of the tritium channel to the ^{14}C channel. All samples were found to exhibit comparable amounts of quenching.

Statistical Analysis

Student's t-test was used to determine the statistical significance of differences in plasma glucose levels between samples (Croxton, 1953). Regression (estimating) equations were calclated for renal data using the method outlined by Croxton (1953). The coefficients of correlation, r, were calculated using the Pearson product-moment formula.

RESULTS

Annual Temperature Changes in Lac Ste. Anne

The annual temperature fluctuation at the 3 m. level in Lac Ste. Anne during 1966 was 19°C (Figure 5a and Appendix Table 3). Water temperature increased steadily during May and June then gradually decreased during August and September. The most rapid decrease in water temperature occurred in October just prior to ice formation on the lake. The highest 3 m. temperature observed in 1966 was 19.7°C. The maximum vertical stratification of temperature observed in the lake was 3°C. Temperatures were taken at the site and depth of the trap net and hence probably represent the temperatures at which the animals used in this study were living. White suckers encounter the most rapid temperature fluctuations during their pre-spawning migration in the tributary stream. Water temperature in the stream could increase from near 0°C in the morning to 6 or 8°C by mid-afternoon.

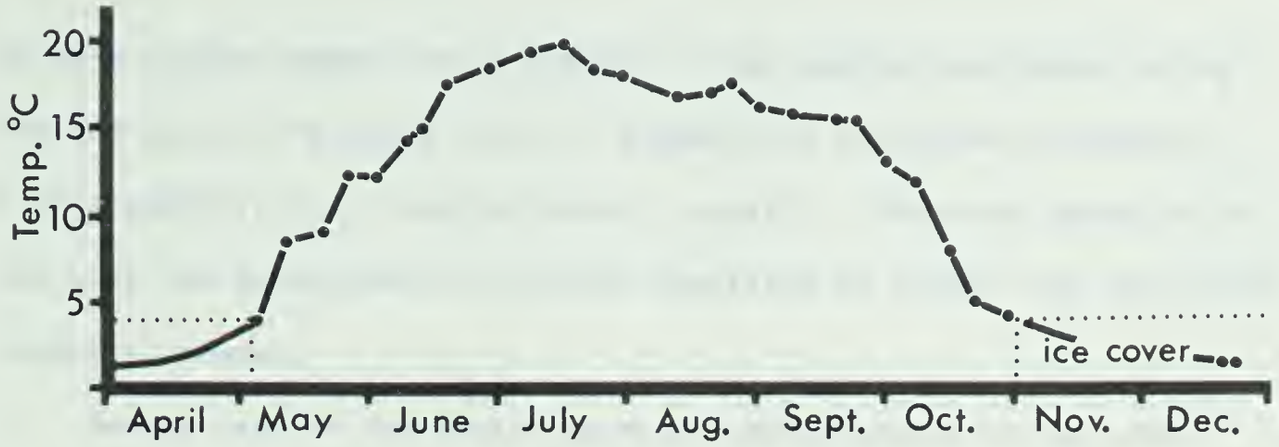
Annual Gonadal Growth in Adult White Suckers

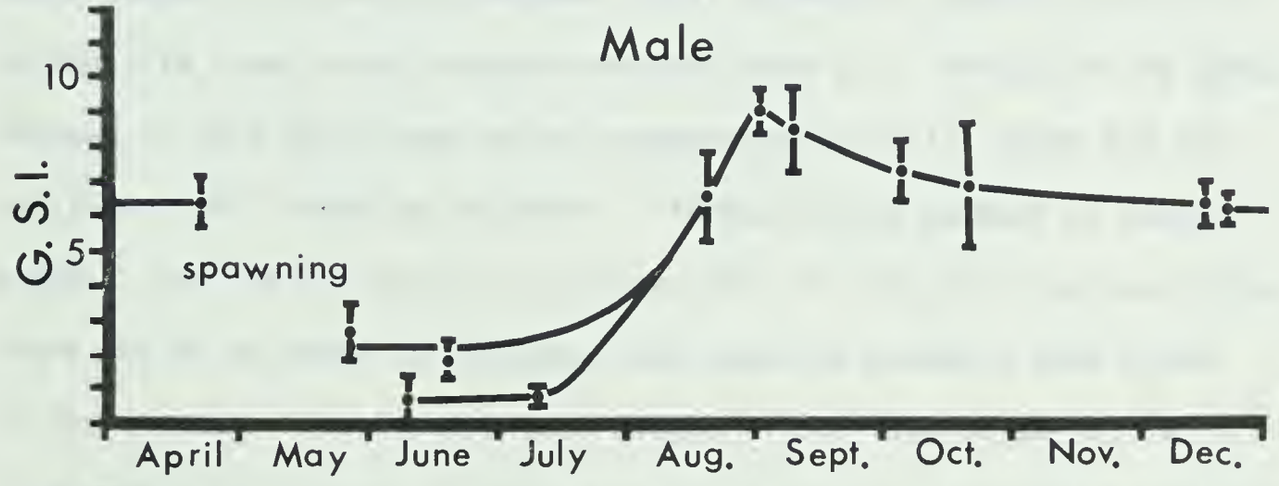
The gonadosomatic index (GSI) used to determine the seasonal changes in gonadal size was the percentage of the total body weight represented by gonad (Fig. 5b and 5c). The male GSI appears to decrease from September to December (Fig. 5b and Appendix Table 4). This decrease in GSI also appeared when the gonadosomatic index used was gonad weight compared to fork length (Appendix Table 4). No evidence was obtained to indicate that the loss was due to sperm leakage. No change in GSI was observed in male fish from mid-December until spawning occurred in May (Appendix Table 4). Two populations

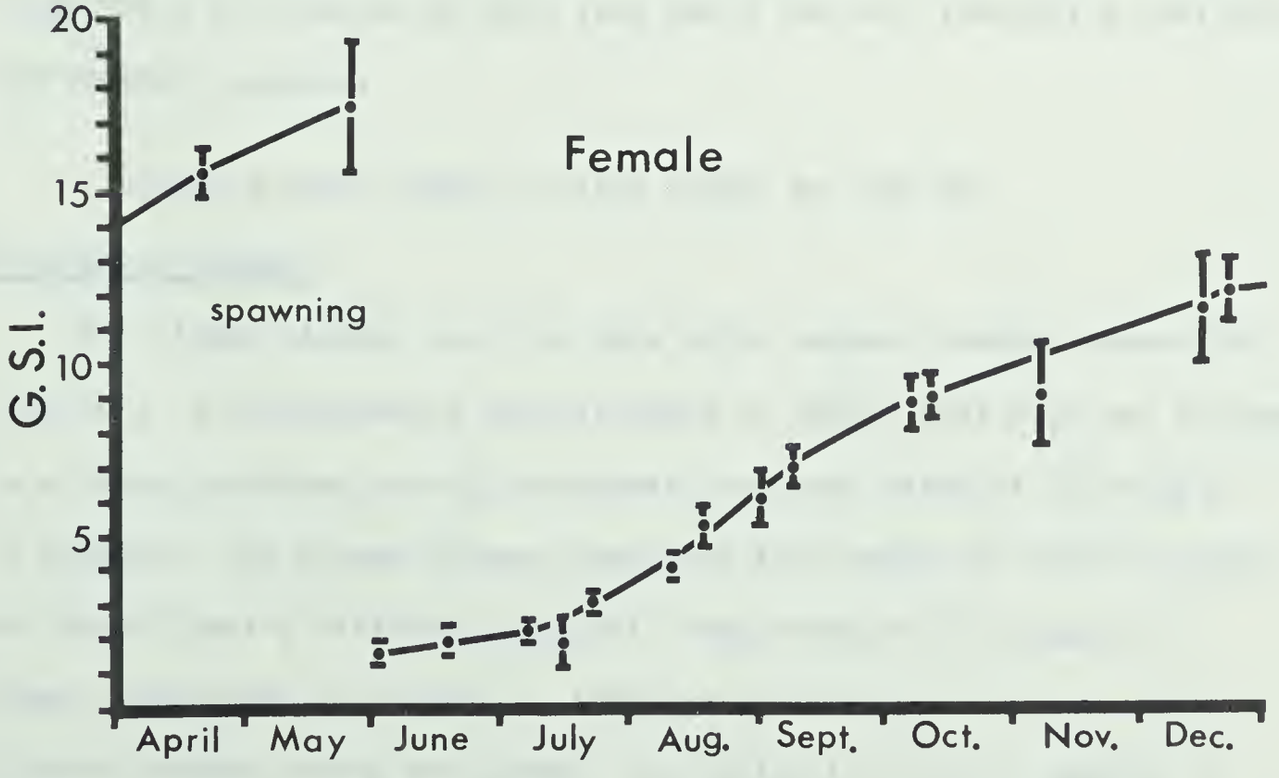
Figure 5. a. The water temperature in Lac Ste. Anne during 1966 taken at 3 m depth at the site of the trap net. The water temperature remained below 2°C from January to April.

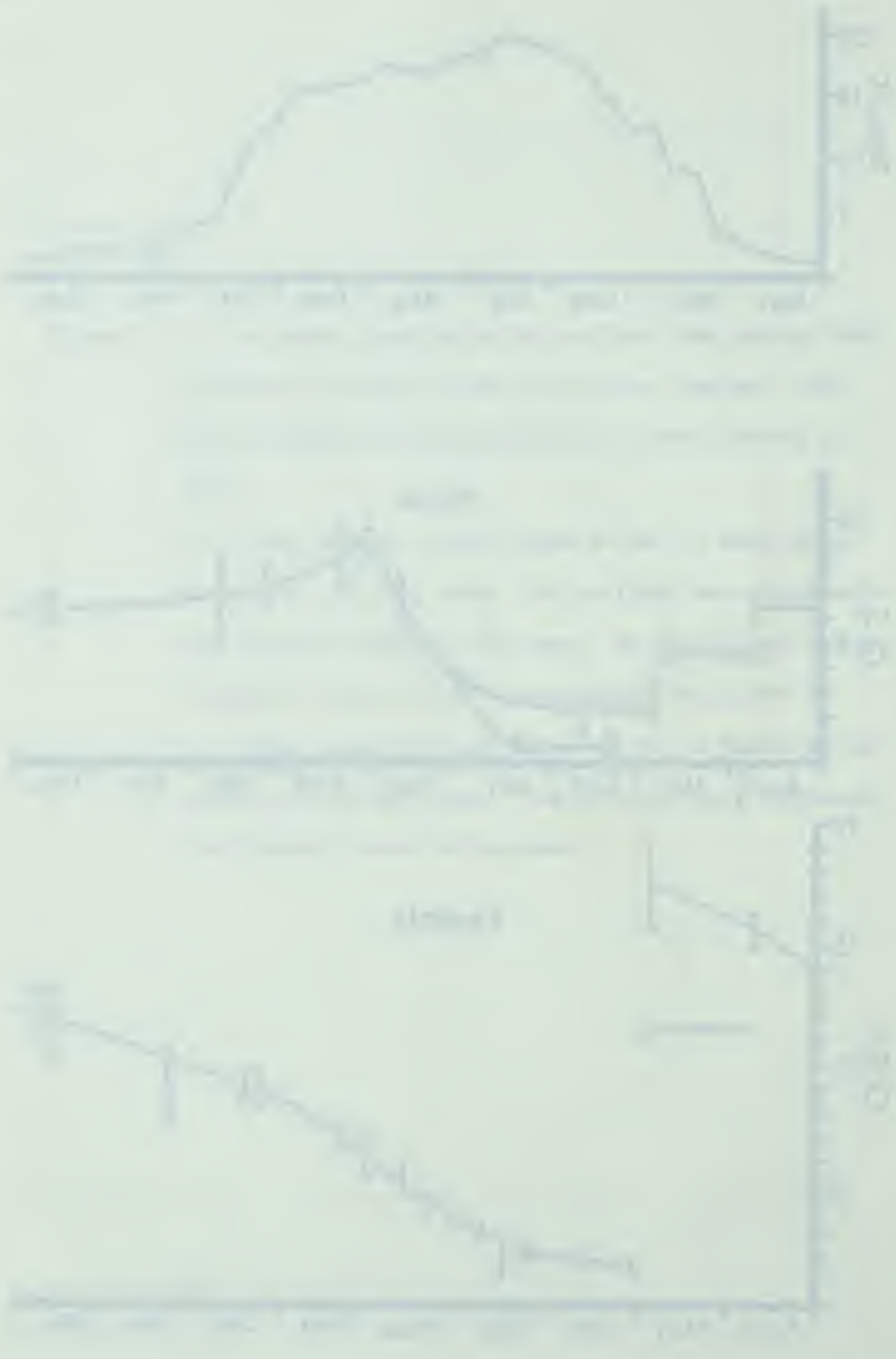
b. Annual gonadal growth based on GSI in male white suckers in Lac Ste. Anne. The vertical bars represent two standard errors of the mean. No significant change in gonadal size occured from December until April.

c. Annual gonadal growth based on GSI in female white suckers in Lac Ste. Anne. The vertical bars represent two standard errors of the mean.


Temp. °C
20
15
10
5
ice cover
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.


Male
G.S.I.
10
5
spawning
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.


Female
G.S.I.
20
15
10
5
spawning
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.



of male suckers appear to be present in the samples collected during May and June. The group with the highest GSI consisted of smaller fish apparently just reaching sexual maturity. The other group which had very low gonadosomatic indicies consisted of larger fish which had recently spawned.

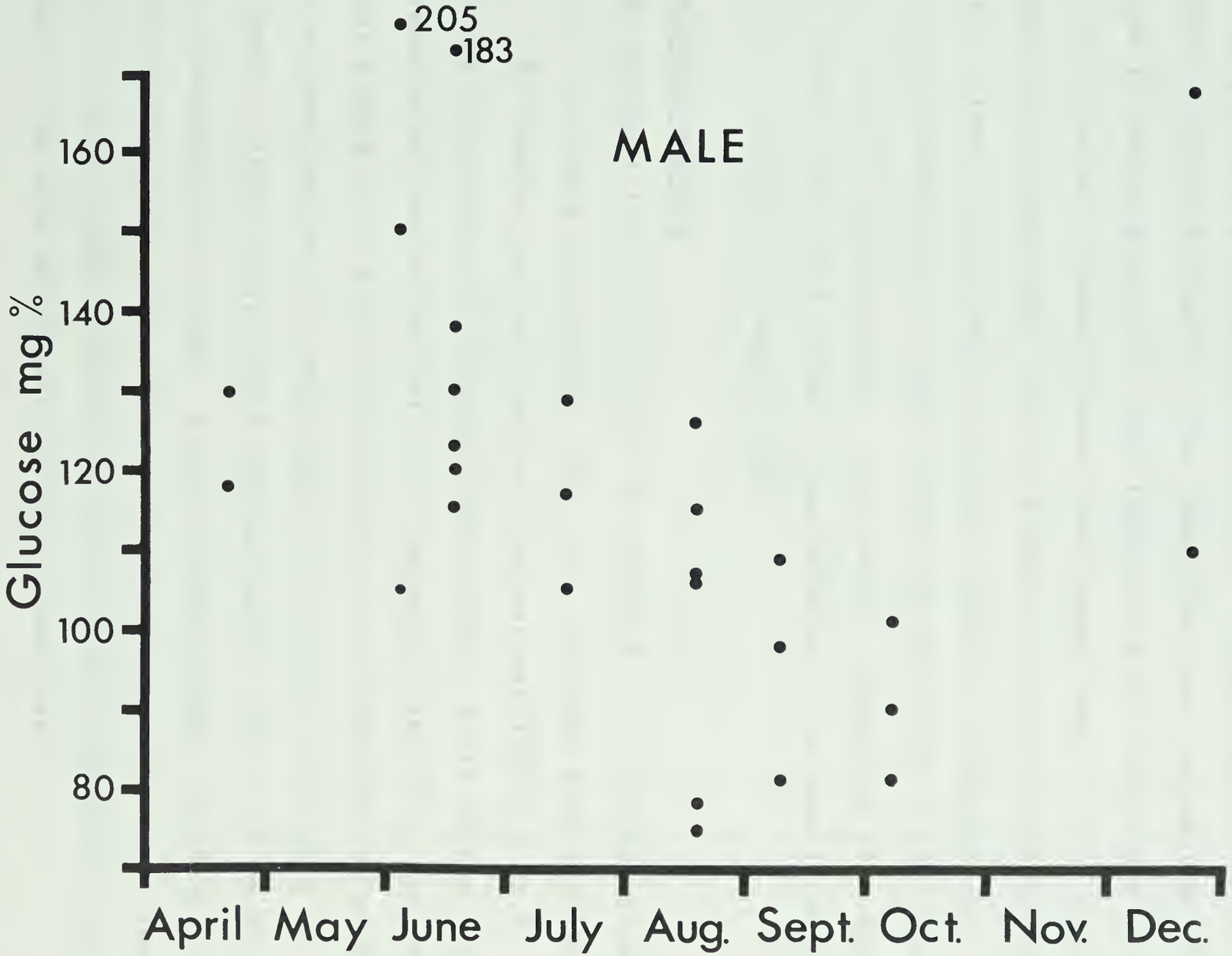
Growth rate of the female gonad proceeds rapidly during late summer and fall, but decreases after the water temperature drops below 2°C (Fig. 5c). The gonadosomatic index for females remained at approximately 12 from mid-December until mid-March (Appendix Table 5). During this time, water temperature was below 2°C. Growth of the gonad resumed in late March when water temperature was still below 4°C and continued until spawning occurred. Although large numbers of suckers migrate into the tributary of Lac Ste. Anne in late April and early May, there was no evidence to indicate that spawning actually took place in the stream. Ripe females were caught in the lake on May 25 but the larger male fish caught at this time had a low GSI, indicating that they had recently spawned.

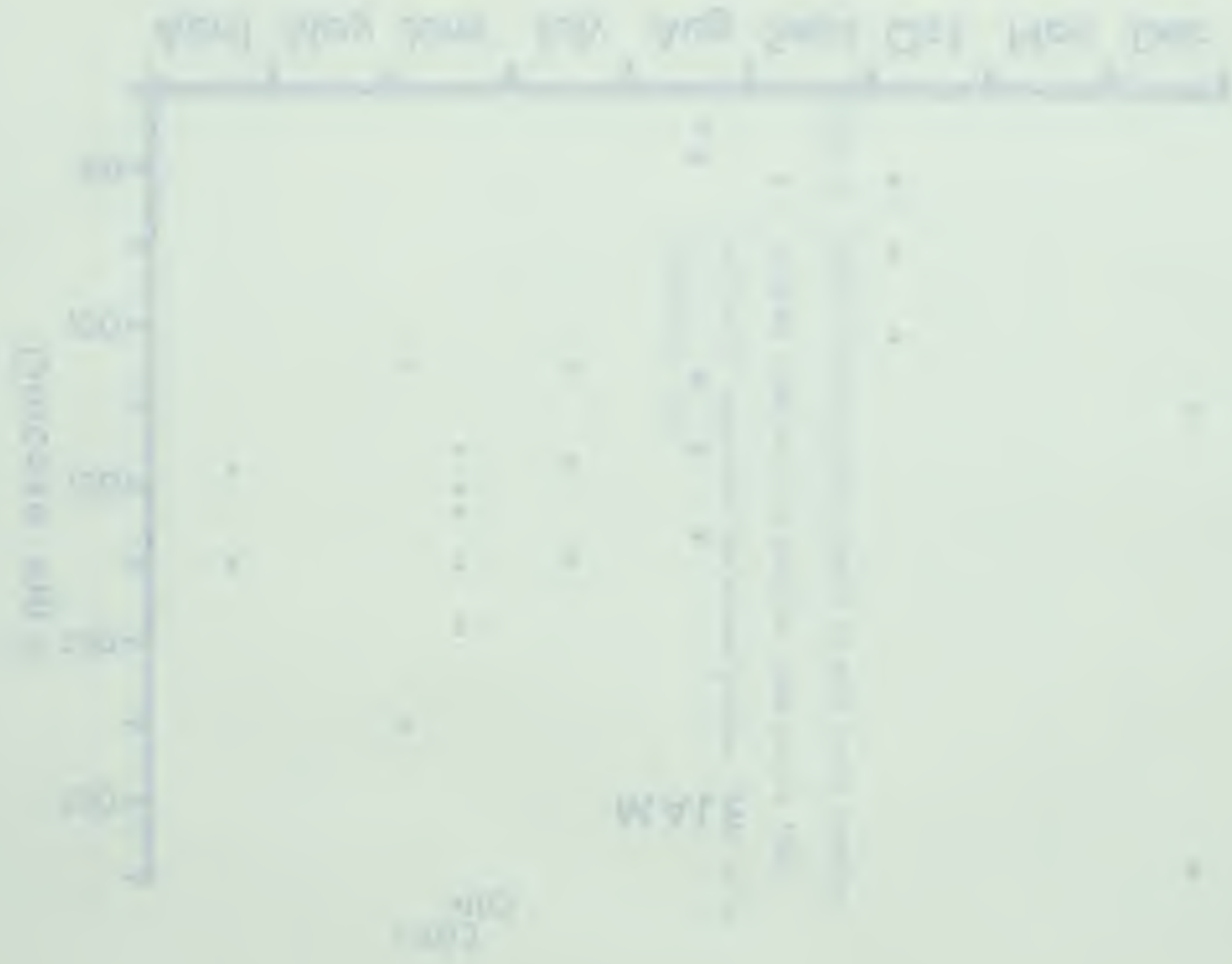
PLASMA GLUCOSE LEVELS OF FISH CAUGHT BY TRAP NET

Male White Suckers

The plasma glucose level of male white suckers changes seasonally (Fig. 6). A post-spawning hyperglycemia of 153 ± 50 (SD) mg.% was followed by a steady decrease during the summer to a mean value of 91 ± 10 mg.% in October. The plasma glucose levels of fish caught in April and June are significantly different ($p<0.001$) from those of fish caught in August, September and October. Although the average level of plasma glucose changes during the summer, the variability within samples is

Figure 6. Normal plasma glucose levels in male white suckers taken by trap net from Lac Ste. Anne during 1966. Each point represents the plasma glucose level of an individual fish.


205
183
MALE
160
140
120
100
80
Glucose mg %
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.



relatively consistent (Appendix Table 6). The average standard deviation of all groups was 22.5 mg.%. Seasonal changes do not appear to directly affect plasma glucose levels. Glucose levels appear to decrease during June when the lake temperature was increasing; then continue to decrease when the lake temperature was relatively constant, (Fig. 5). The male plasma glucose levels were lowest during late September and October when the lake temperature was decreasing most rapidly. Since only two male fish were sampled in December, no conclusive statement can be made concerning the normal plasma glucose level during the winter months. The seasonal changes in male glucose levels appear to be more closely associated with the sexual cycle than with environmental temperatures.

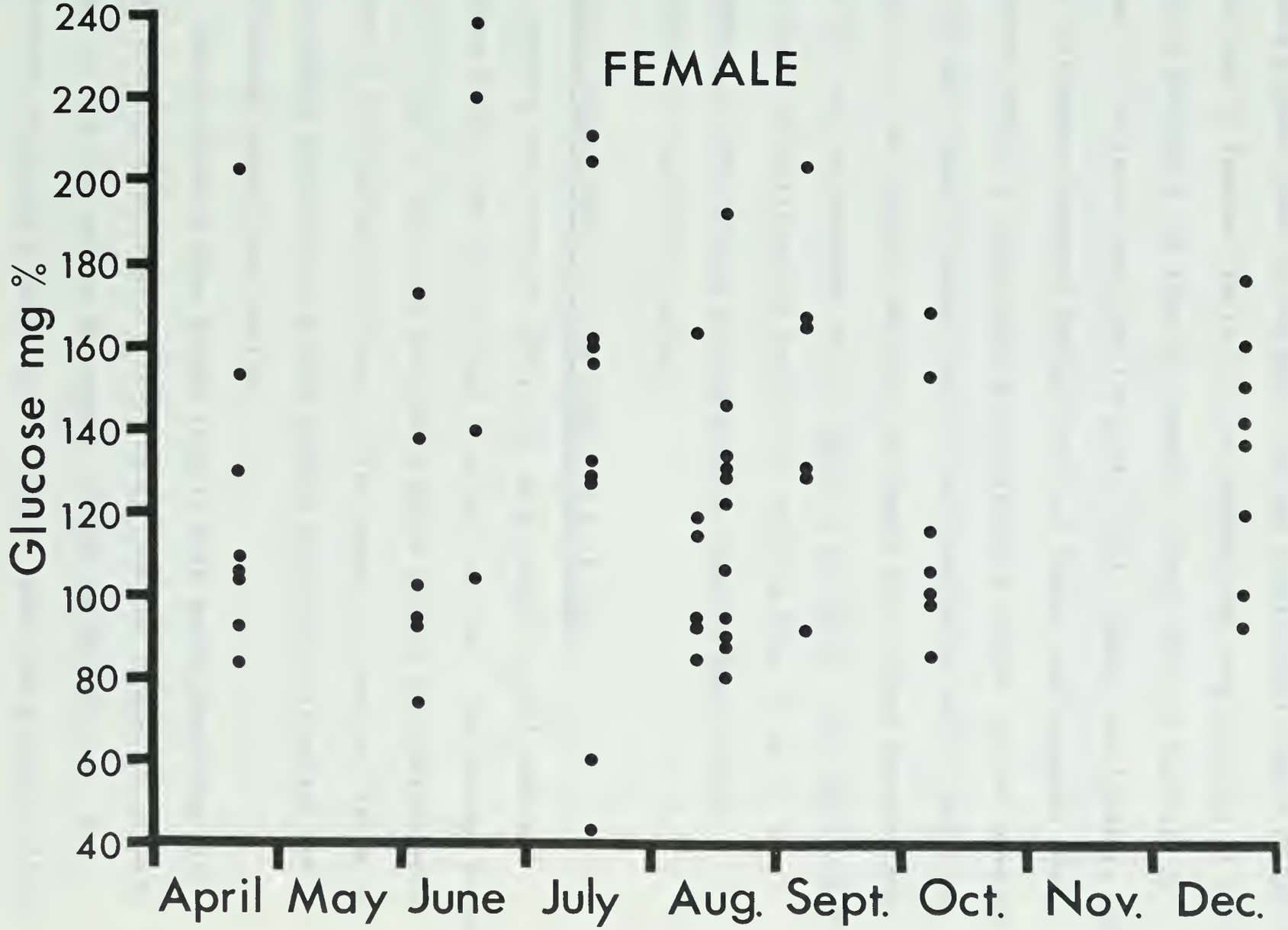
Female White Suckers

No seasonal fluctuation could be detected in the plasma glucose level of the female white sucker (Fig. 7). The mean plasma glucose level of 66 females sampled from the trap net was 129 ± 40.3 mg.%. The standard deviation of plasma glucose levels of all female fish was more than twice that of the male fish (Appendix Table 6). Any small seasonal change which might have occurred in the females was masked by the extreme variability which occurred between individuals in each sample. The average female plasma glucose level (123 mg.%) during August, September and October is significantly higher ($0.005<p<0.01$) than the average male plasma glucose level (97 mg.%) over the same period. At other times, however, no significant difference could be detected in the male and female plasma glucose levels.

Plasma Glucose Levels of Northern Pike Taken From the Trap Net

Northern pike were caught in the trap net in sufficient numbers

Figure 7. Normal plasma glucose levels in female white suckers taken by trap net from Lac Ste. Anne during 1966. Each point represents the plasma glucose level of an individual fish.


FEMALE
Glucose mg %
240
220
200
180
160
140
120
100
80
60
40
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.

for sampling only during June and July. At that time, the average plasma glucose level of the male fish was significantly lower ($0.025<p<0.05$) than that of females (Table 1). Not enough pike were captured at Lac Ste. Anne to determine the time of spawning. These fish are reported to spawn in the spring when the ice melts (Carl, Clemens and Lindsey, 1958). The differences observed between male and female pike suggests that females undergo a post-spawning hyperglycemia similar to that observed in the male sucker; however, this is not conclusive due to restricted sampling. The standard deviation of female pike plasma glucose levels was 92.0 mg.% as compared to 51.1 mg.% in the males. The magnitude of the standard deviation was greater in the pike than it was in the suckers, but the females of both species showed a much greater variability than did the males.

Plasma Glucose Levels of Fish Caught in Gill Nets

During the winter of 1965, fish were caught in gill nets and sampled in the same way as those from the trap net. The average plasma glucose level of both male and female white suckers was considerably higher in gill-netted fish than in fish caught by trap net (Table 1). The standard deviation of plasma glucose levels of gill-netted and trap-netted suckers was similar.

Female northern pike caught in gill nets during the winter had lower blood glucose levels (122 ± 51 mg.%) than the females which were caught by the trap net in the summer (210 ± 92 mg.%)(Table 1). The difference in plasma glucose levels between these two groups of fish is probably significant ($0.05<p<0.10$). No such difference was detected in male pike. No attempt was made to catch northern pike

Table 1. *Plasma glucose levels of white suckers and northern pike from Lac Ste. Anne.*

SAMPLE	SEX	DATE	n	$\bar{X}$ mg%	σ_x	$\sigma_{\bar{x}}$	RANGE mg%
Pike from Trap Net	F	June-July 1966	9	210	92.0	30.7	47-330
	M		11	131	51.1	15.4	59-240
Pike from Gill Net	F	Dec.-March 1966	5	122	50.8	22.7	78-199
	M		7	124	60.6	22.9	63-210
Suckers from Trap Net	F	April-Dec. 1966	66	129	40.3	5.0	44-238
Suckers from Gill Net	F	Oct.-Dec. 1965	21	183	90.6	19.8	75-383
	M		16	190	84.7	21.2	91-348
Suckers in lab.	F	May '66 to Jan. 1967	8	214	126.7	44.88	90-399
	M		5	176	116.0	52.0	75-389

Symbols used: n = number of individuals in the sample, $\bar{X}$ = mean of sample, σ_x = standard deviation of sample and $\sigma_{\bar{x}}$ = standard error of mean.

by gill net in June or July. The variability in plasma glucose level of all the pike caught by gill net was similar to that of the male pike caught in the trap net.

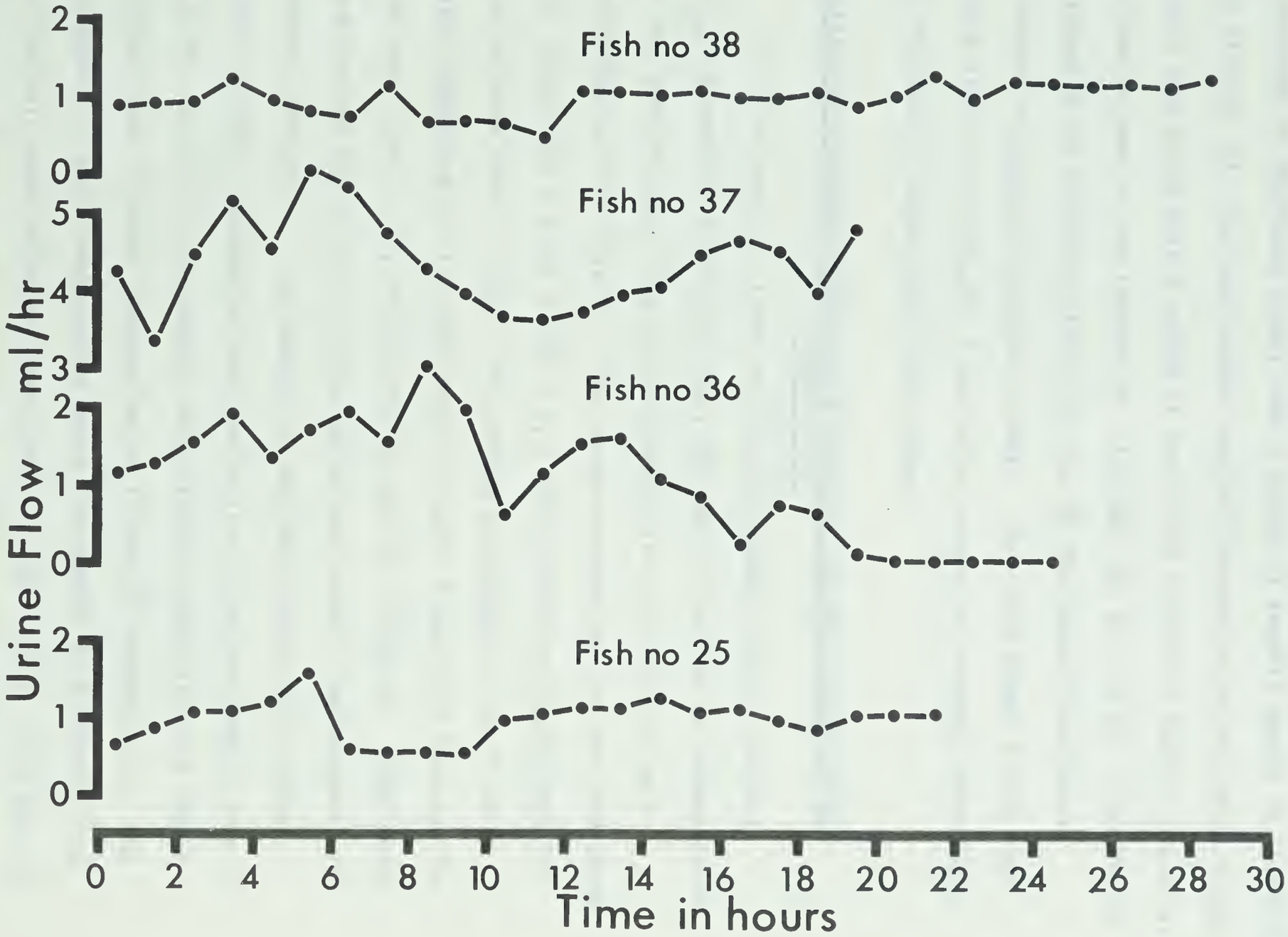
Plasma Glucose Level of Fish Held in the Laboratory

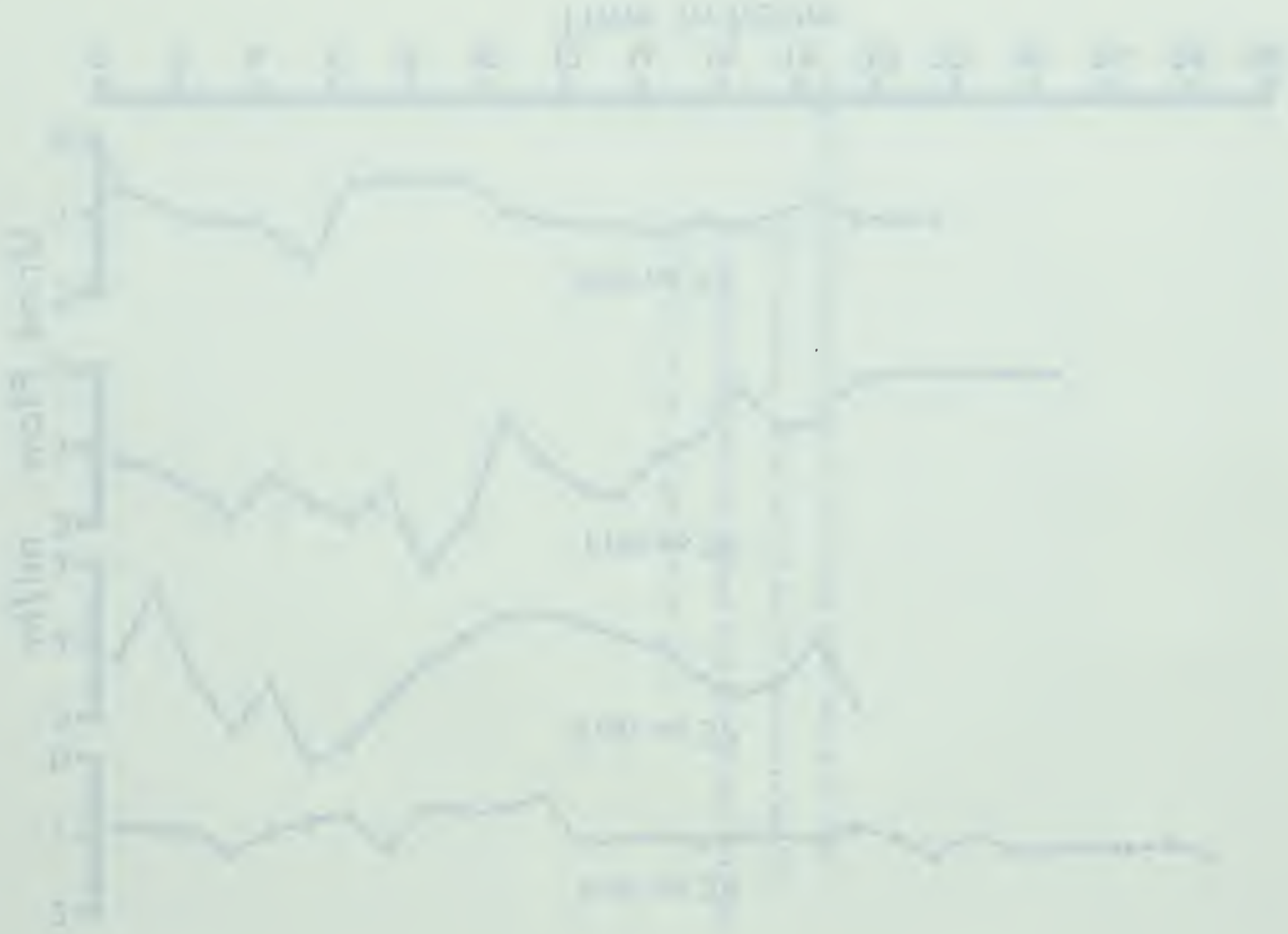
Plasma glucose levels were determined for thirteen of the white suckers used for renal studies. The fish were not disturbed for at least twenty-four hours following cannulation. Blood samples were taken through the cannula without handling the fish. The mean plasma glucose level of these fish was 199 ± 117 mg.% which is slightly higher and much more variable than that of all suckers from gill nets (186 ± 87 mg.%), but the two values are not significantly different. Plasma glucose levels of both the fish sampled by gill net and those sampled in the laboratory were much higher and considerably more variable than those of female fish from the trap net (129 ± 40 mg.%) (Table 1).

Variations in the Urine Flow of Individual Fish

Experimental fish struggled sporadically for twelve to twenty hours after being placed in the leucite boxes following operative procedures. Thereafter, the white suckers remained quiet in the boxes for the duration of the sampling period. Urine flow ceased for a period of several hours following operative procedures. This effect is the opposite of the laboratory diuresis observed in marine fish (Forster, 1953). Even when temperature and activity level were constant, the rate of urine formation by individual white suckers changed markedly over a period of from one to several hours (Fig. 8). In some instances, urine flow was uniform for periods of several hours, as can be seen in the records for fish Nos. 38 and 25. Often urine flow

Figure 8. Selected portions of the urine flow record of four white suckers. Fish No. 38 and 37 were held at 4°C throughout the interval shown; fish No. 36 was held at 6°C and fish No. 25 was held at 8°C.


Fish no 38
Fish no 37
Fish no 36
Fish no 25
Urine Flow ml/hr
2
1
0
5
4
3
2
1
0
2
1
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time in hours

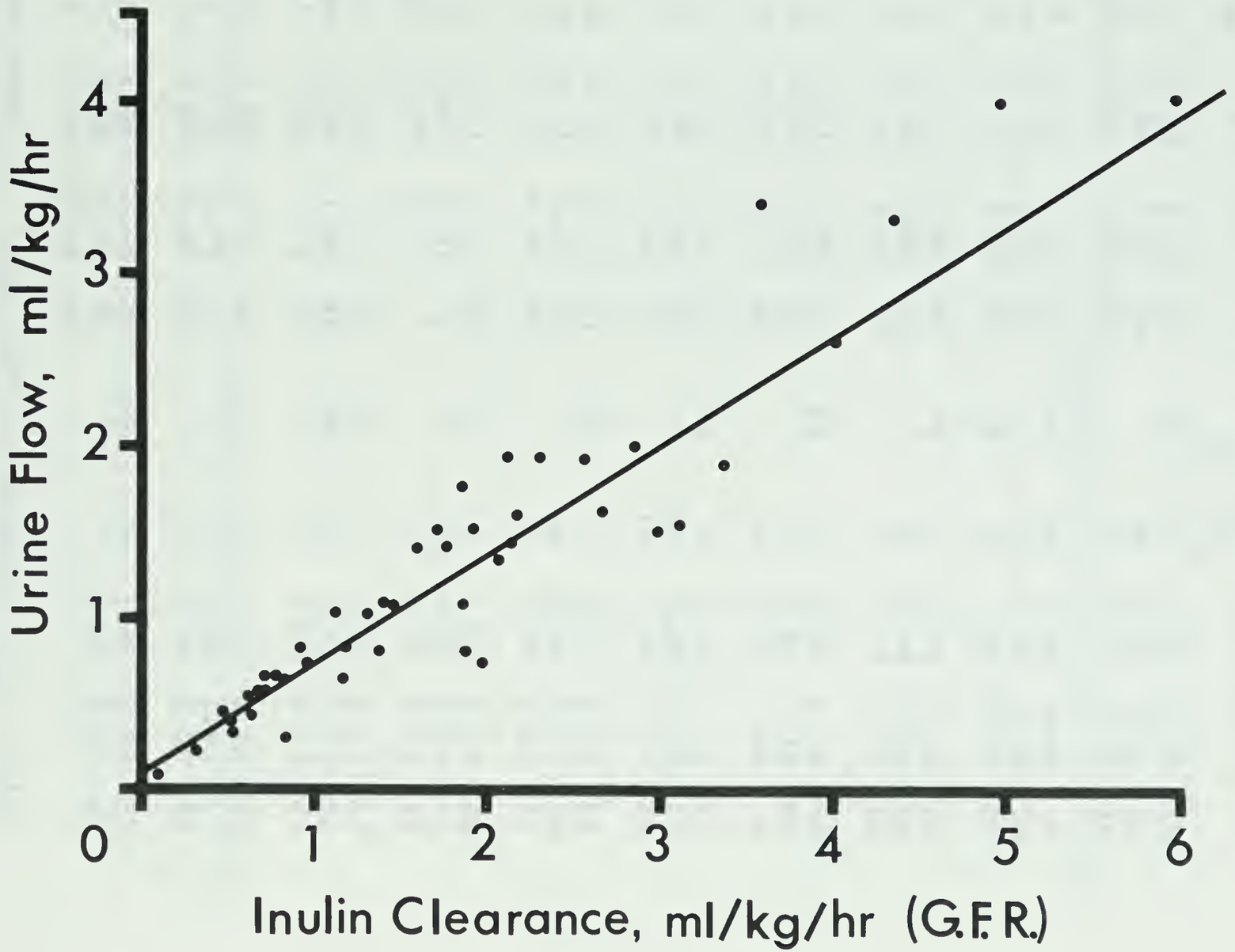


increased or decreased slowly over a period of several hours, as illustrated by fish No. 37. The most common type of fluctuation was an abrupt change which took place over a period of one or two successive hourly intervals, as can be seen in the records for fish Nos. 25, 36, and 37. Fish No. 36 stopped urine production (hour 20, Fig. 8) for twenty-three hours (without any apparent external stress). This fish showed a very high urine flow (4.9 ml./kg./hr.) when urine production resumed, presumably to re-establish normal body fluid volume.

Relationship Between Glomerular Filtration Rate and Tubular Water Reabsorption

In this study, a linear relationship between inulin clearance (C_{in} = glomerular filtration rate, GFR) and urine flow was demonstrated for the combined data from nine different fish, all of which were maintained at 8°C (Fig. 9). The regression equation for the line shown is: Urine flow = $0.102 + 0.64 \times C_{in}$; the correlation coefficient (r) for the data shown is 0.92. The average percentage of the filtered water reabsorbed for the data shown in Figure 9 was 29.3% (average for fish Nos. 3, 4, 7, 12, 16, 25, 26, 28, and 29) (Table 2). Experiments were carried out on pairs of fish which were caught simultaneously and treated identically. The members of each pair showed very similar rates of water reabsorption over the entire filtration range (Table 2). Fish No. 28 and 29, for example, were treated identically and showed average rates of water reabsorption of 19 and 25% respectively. The difference in water reabsorption among fish caught at different times or fish held under different conditions was significant. Fish Nos. 39 and 40 for example showed average water reabsorption of 52 and 47% respectively, while fish Nos. 25 and 26 showed 35 and 28% water

Figure 9. Relationship between inulin clearance (GFR) and urine flow in the white sucker. All the data was obtained at 8°C, 36 of the 45 points shown were obtained from fish Nos. 25, 26, 28, and 29. The other 11 points were obtained from fish Nos. 3, 4, 7, 12, and 16.


Urine Flow, ml/kg/hr
4
3
2
1
0
1
2
3
4
5
6
Inulin Clearance, ml/kg/hr (G.F.R.)

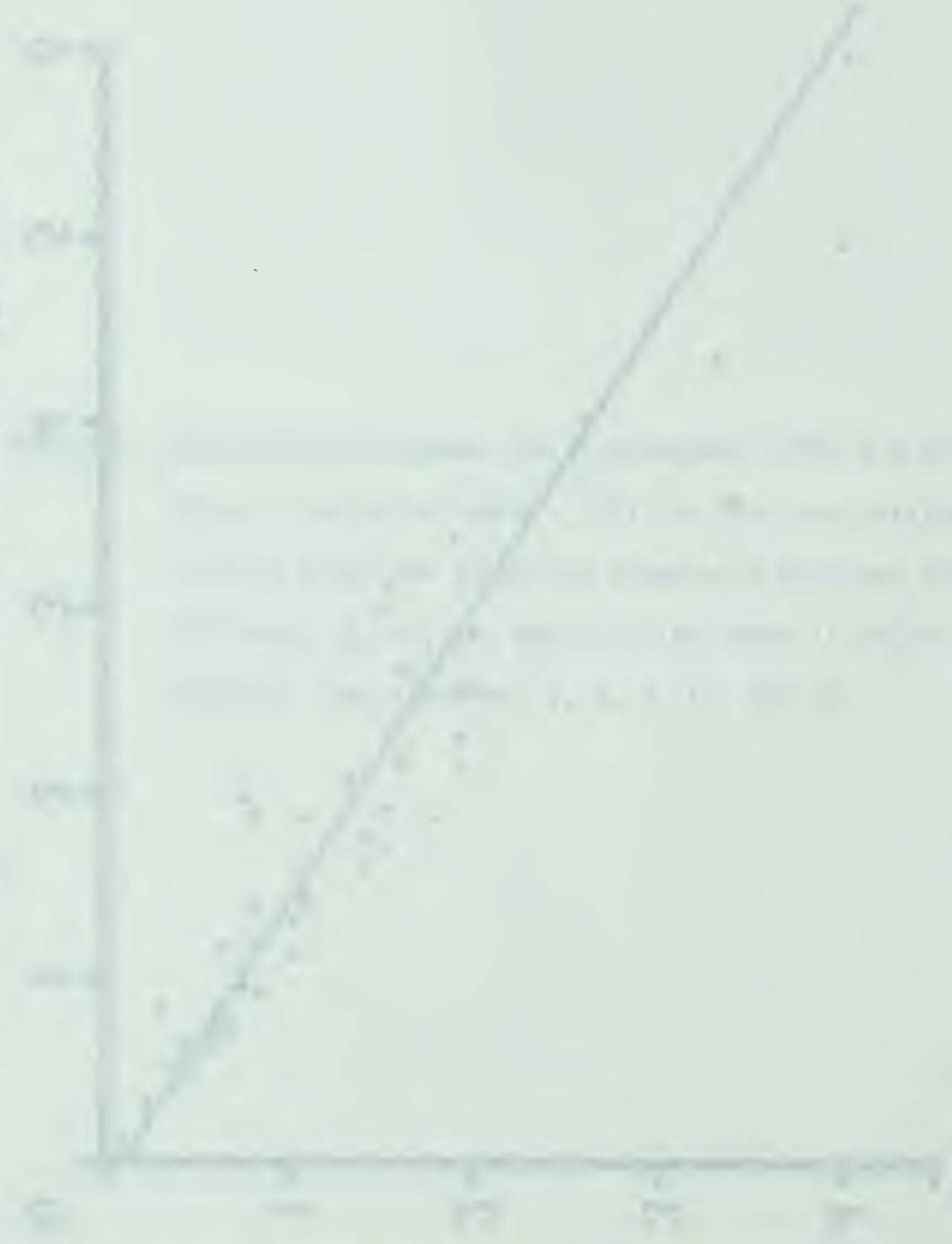


Table 2. The relationship between filtration rate and urine flow in the white sucker.

Fish #	Sam. #	U	C_{in}	% R	Fish #	Sam. #	U	C_{in}	% R
3	7	3.95	4.99	21	28	7	0.67	0.77	13
"	10	2.69	4.07	34	"	8	0.53	0.62	14
"	14	3.98	5.99	33	"	9	1.02	1.13	10
4	4	0.42	0.62	33	28	10	0.45	0.48	6
"	6	0.73	1.97	63	"	11	0.73	0.97	24
7	4	2.01	2.86	30	29	2	1.60	4.41	64
7	6	1.51	2.98	49	29	3	1.54	1.94	21
12	2	1.35	2.07	35	"	4	1.94	2.56	24
16	2	3.42	3.58	4	"	5	1.51	1.74	14
25	2	0.81	1.90	57	29	6	1.93	2.30	16
"	3	0.58	0.72	19	"	7	1.60	2.16	26
"	4	0.57	0.73	22	"	8	1.76	1.86	6
25	5	0.07	0.11	39	29	9	1.44	1.62	11
"	6	1.09	1.42	24	"	10	1.00	1.32	23
"	7	0.39	0.54	27	"	11	1.90	3.37	44
25	8	0.35	0.52	33	39	1	1.11	2.43	54
"	9	0.31	0.62	50	"	2	1.24	2.77	55
26	3	3.32	4.36	24	"	3	1.36	2.94	35
26	4	0.83	1.02	19	39	4	1.66	3.17	48
"	8	0.83	0.91	8	"	5	2.17	3.78	42
"	12	0.66	0.71	7	"	6	2.79	4.31	35
26	13	1.55	3.11	50	39	7	1.79	3.32	40
"	14	1.09	1.86	41	40	1	0.33	0.75	56
"	15	1.64	2.69	39	"	2	1.60	4.33	63
26	16	0.80	1.38	42	40	3	1.37	2.50	45
28	2	1.43	1.76	19	"	4	0.63	1.91	67
"	3	0.66	0.82	20	"	5	1.59	3.16	50
28	4	1.09	1.45	25	40	6	1.60	2.38	33
"	5	0.23	0.32	27	"	7	0.11	0.21	48
"	6	1.43	2.14	33					

Symbols used: U = urine flow in ml/kg/hr, C_{in} = inulin clearance (GFR) in ml/kg/hr, % R = percentage of the filtered water reabsorbed.

reabsorption respectively. Fish Nos. 39 and 40 were caught in January and held for 4 days at 4°C. Fish Nos. 25 to 29, however, were caught on July 22 and were held in the laboratory for 10 to 13 days at 8°C.

Relationship Between Glomerular Filtration Rate and Glucose Transport Maximum

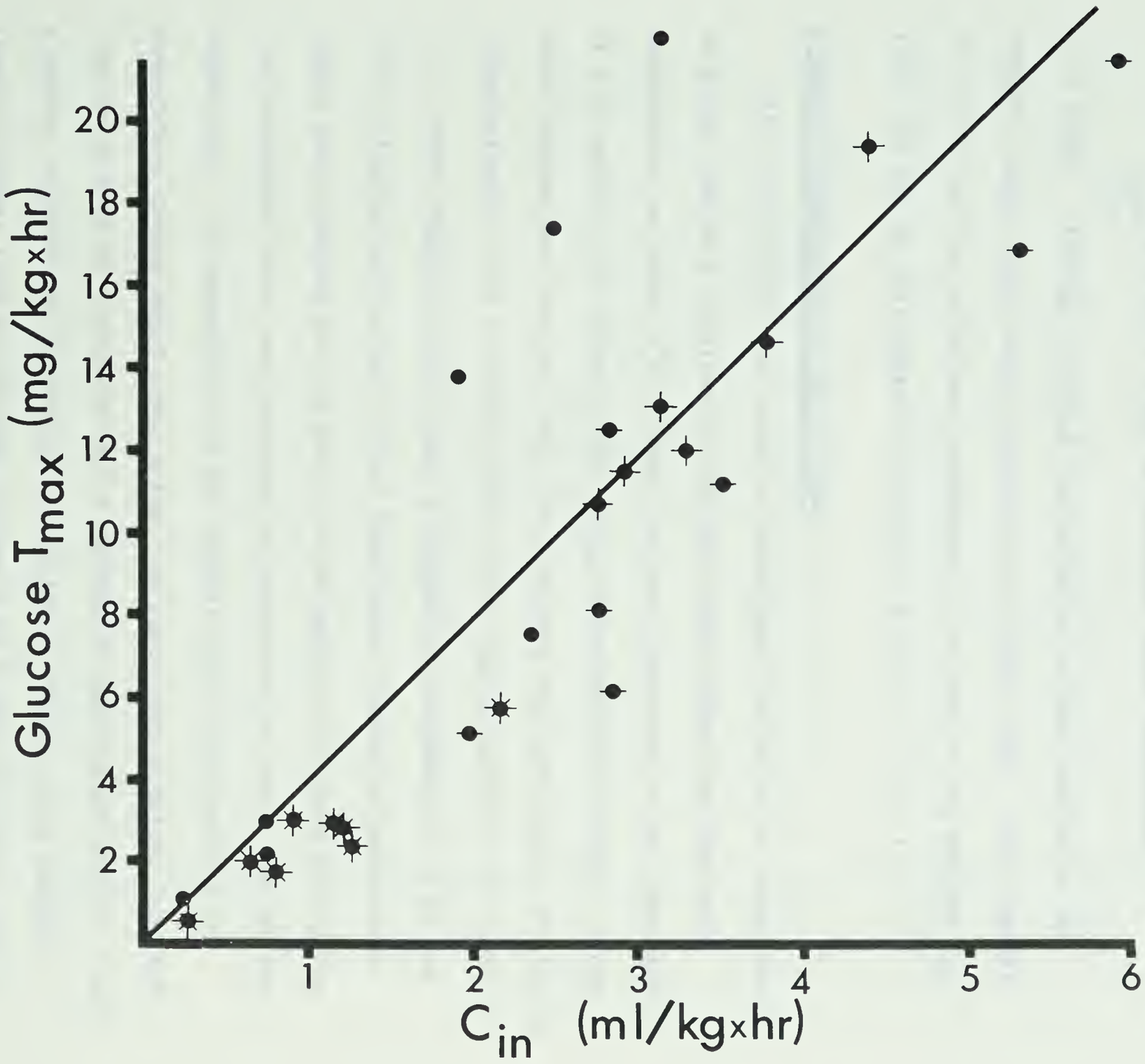
A linear relationship can be demonstrated between glomerular filtration rate (C_{in}) and glucose transport maximum (Tm_G) over the range of filtration rates investigated (Fig. 10). The regression equation for the data shown in figure 10 is: $Tm_G = -0.194 + 3.881 \times C_{in}$; and the correlation coefficient, r, for the data shown is 0.86. Much variation existed among individual fish as can be seen in Figure 10. Variation also existed within individuals. For example, fish No. 40 shows two distinctly different linear relationships between Tm_G and C_{in}. Some of the observed variation in fish No. 40 could be the result of the infusion and sampling procedure used. If the double cannula had been in the artery, the infusate would be carried back by the blood flow to the sampling portion of the cannula, thus giving an erroneous value for the plasma glucose level.

Relationship Between Tm_G/C_{in} And The Percentage Of Water Reabsorption

Since a linear relationship existed between Tm_G and C_{in}, the ratio of Tm_G to C_{in} will be constant and independent of filtration rate if glomerular intermittency alone is responsible for variations in filtration rate.

When the variation in Tm_G, which is due to changes in C_{in}, is eliminated by dividing Tm_G by C_{in}, a relationship is found to exist between the percentage of filtered water which is reabsorbed and the

Figure 10. Relationship between inulin clearance (C_{in}) and glucose transport maximum (Tm_G). Data was obtained at 4°C. The symbols shown represent: ✷ fish No. 34, ✦ fish No. 39, ● fish No. 40 and ✦ fish Nos. 3, 6, 7, 12, 16, and 18.


Glucose T_{max} (mg/kg×hr)
20
18
16
14
12
10
8
6
4
2
1
2
3
4
5
6
C_{in} (ml/kg×hr)

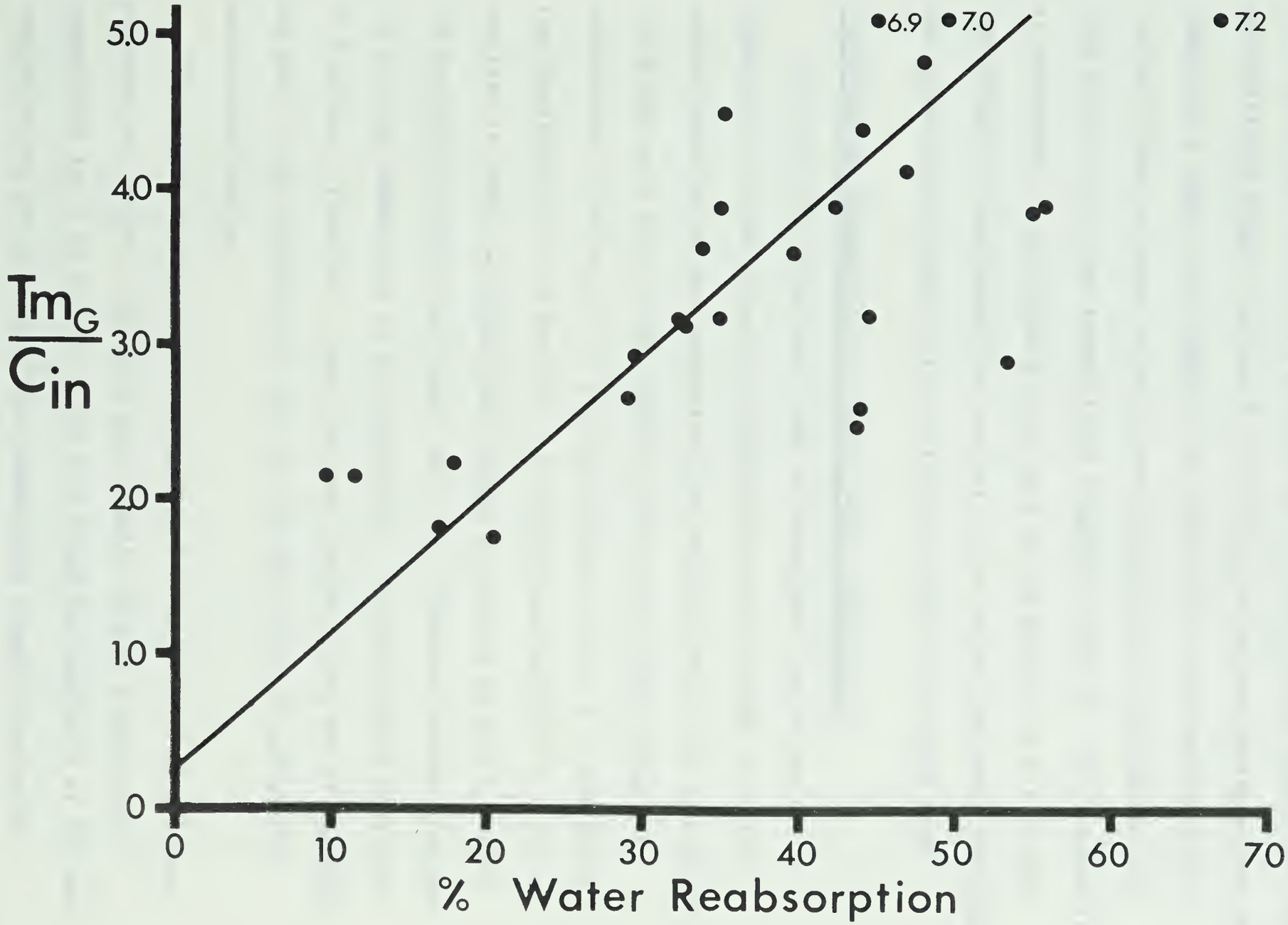
quantity of glucose which is reabsorbed per millilitre of filtrate formed (Fig. 11). The regression equation which describes the data shown is: $\frac{Tm_G}{C_{in}} = 0.254 + 0.089 \times$ (per cent water reabsorbed). ($r = 0.76$). Although the data are variable, no more variation was observed among individuals than within individual fish. Over one-half of the variability which was encountered in a plot of Tm_G against C_{in} (Fig. 10) can be attributed to the data shown in Fig. 11.

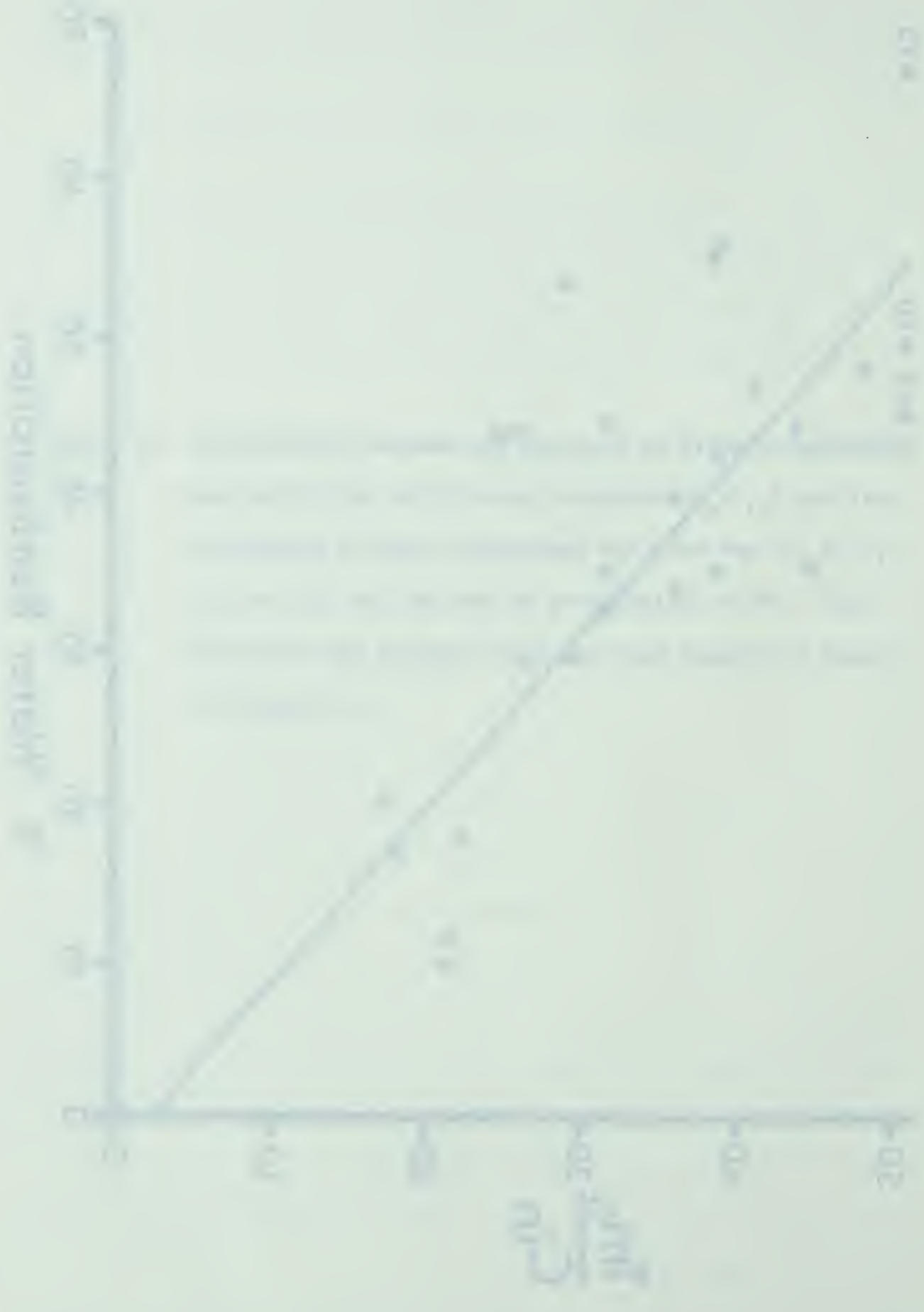
Effect of Temperature on Urine Flow

All of the eight fish used to investigate the effect of temperature on urine flow were obtained in fall and winter, when the lake water temperature was below 4°C. These fish were then held in the laboratory at 4°C, usually for three or four days, before catheterization. Since blood samples were not required, the fish were not cannulated. Following catheterization, the fish were held in the leucite boxes for 24 hours at 4°C. Water temperature was usually changed by increments of 2°C; the urine collected during the initial three hours following temperature change was discarded and the hourly urine samples collected during the nine subsequent hours were averaged to determine the urine flow at that temperature. To compensate for any effects which the direction of temperature change might have, urine flow rates were obtained from each fish using both an increasing and a decreasing temperature regime. Such a regime required the continuous collection of hourly urine samples from individual fish for periods of from 5 to 8 days depending on the temperature range investigated.

Urine flow in the white sucker increased with rising temperature

Figure 11. Relationship between the quantity of glucose reabsorbed per millilitre of filtrate produced (Tm_G/C_{in}) and the percentage of water reabsorbed for fish Nos. 3, 6, 7, 12, 16, 18, 34, 39, and 40 all sampled at 4°C. The data used was obtained from the same samples as those in Figure 10.


6.9
7.0
7.2
5.0
4.0
3.0
2.0
1.0
0
$\frac{Tm_G}{C_{in}}$
0
10
20
30
40
50
60
70
% Water Reabsorption



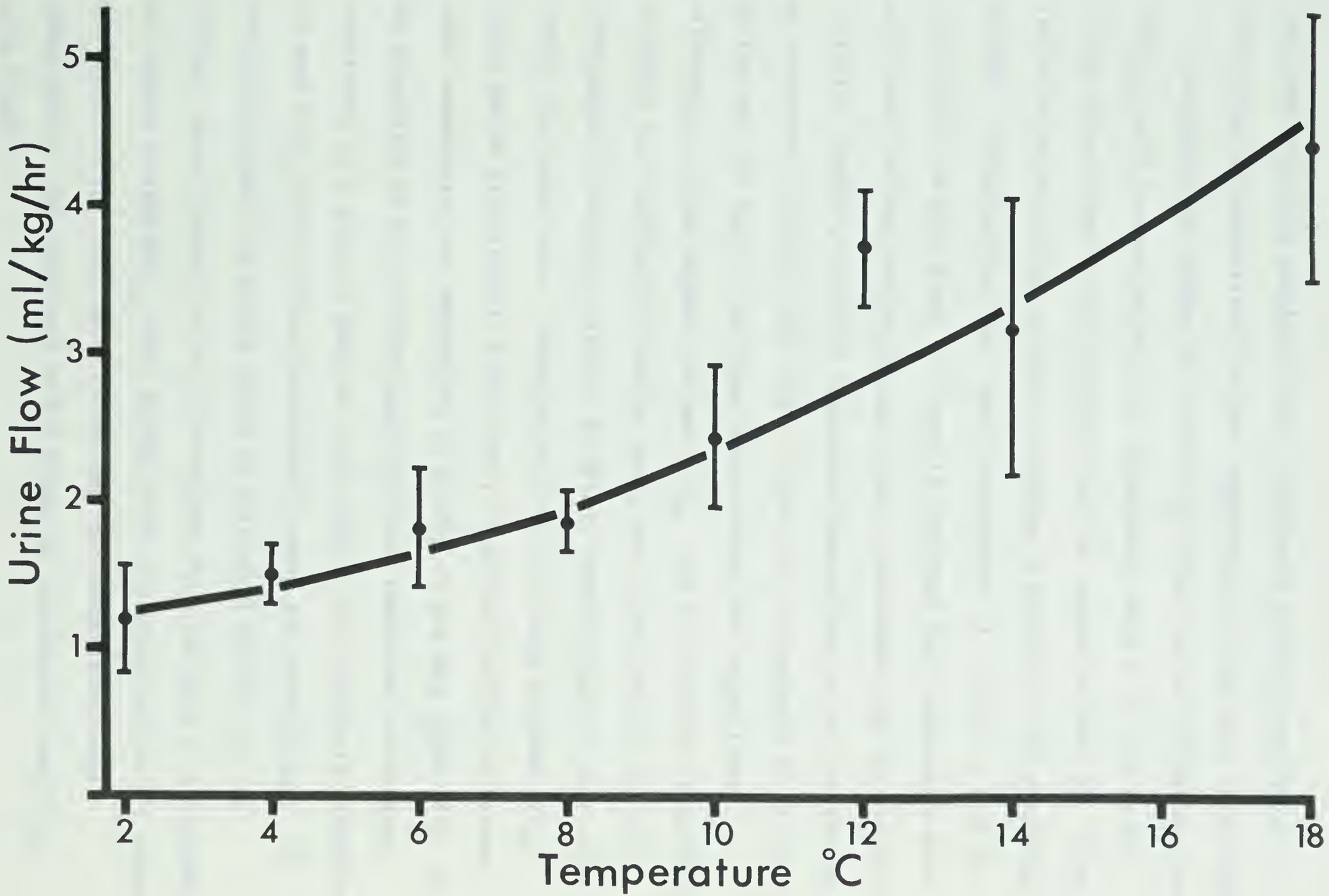
over the range of 2°C to 18°C (Fig. 12). The Q_{10} value, determined from an eye-fitted smooth line was 2.2. This Q_{10} value was constant over the temperature range investigated. The urine flow rates were characteristically more variable at the higher temperatures investigated than they were at the lower temperatures (Appendix Table 8). This variability is reflected in the standard error bars on the graph. At all of the temperatures investigated, the variability in urine flow among individuals was approximately equal to the variability within individual fish.

The Effect of Temperature on Tubular Water Reabsorption

The nine fish which were used to investigate the effect of temperature on tubular water reabsorption can be divided into three groups according to the temperature and holding time in the laboratory. Two groups were held in the laboratory for thirty days; group one consisted of fish Nos. 3, 4, and 5 which were held at 2°C, and group two, consisting of fish Nos. 6, 7, 8, and 9, was held at 4°C. All of the fish in these two groups were caught at the end of April, during the prespawning migration; hence, the only difference between them was the holding temperature in the laboratory. The third group consisted of fish Nos. 33 and 34. These fish were caught at the end of September and held in the laboratory at 8°C for ten days prior to cannulation and catheterization.

The water temperature was changed by increments of 2 to 4°C over a period of 2 hours. Fish were then usually held at a particular temperature for 2 or 3 hours. The fish which had been held at 8°C were sampled at the 2°C portion of the temperature range following the sampling at 8°C; then samples were taken at the higher temperatures.

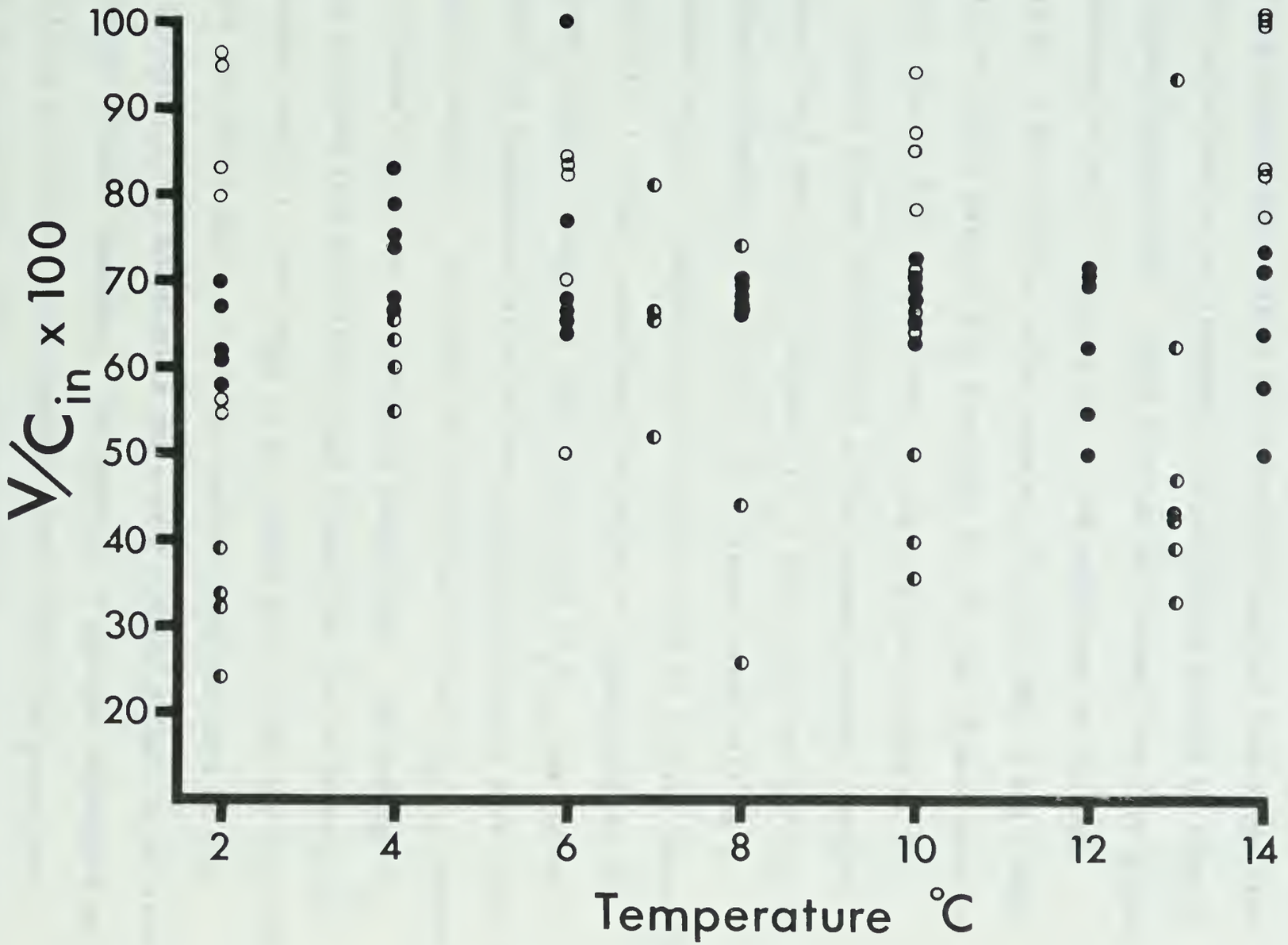
Figure 12. The effect of temperature on urine flow in the white sucker. The means are calculated from the average urine flow, over nine-hour intervals, of several fish. The vertical bars represent two standard errors of the mean.


Urine Flow (ml/kg/hr)
5
4
3
2
1
2
4
6
8
10
12
14
16
18
Temperature °C

The other fish were sampled at their laboratory holding temperature first, then at progressively higher temperatures of the range investigated.

No significant change in the ratio of urine flow to filtration rate (V/C_{in}) could be detected over the temperature range 2 to 14°C in any of the three groups of fish studied (Fig. 13. Appendix Table 9). Since the ratio of urine flow to inulin clearance is related to the percentage of water reabsorbed (per cent water reabsorbed = $(1 - V/C_{in}) \times 100$) by the tubule, the data shown in Figure 13 indicates that temperature does not directly affect the percentage of water reabsorbed from the glomerular filtrate. However, increased temperature does affect urine flow through an increased filtration rate (Figs. 12 and 13). An analysis of variance on the data for each of the three groups showed that significant differences existed between the mean V/C_{in} ratio at different temperatures for group one ($0.05<p<0.10$) and for group two ($0.001<p<0.01$); but the differences in the V/C_{in} ratio at different temperatures was not significant ($p>0.1$) for group three. Although the variation among the mean V/C_{in} ratios may be statistically significant, no general correlation between water reabsorption and temperature is evident in the data shown (Fig. 13). The proportion of the filtered water which was reabsorbed varied among individuals of a group as much as it did within the individuals themselves. The mean V/C_{in} ratio differed considerably among the three groups. The difference between the groups cannot be attributed entirely to the previous thermal history of the fish because the group held at 8°C showed the highest average V/C_{in} ratio (0.80), while the group held at 4°C showed the lowest average V/C_{in} ratio (0.49) over the temperature range investigated. The group held at 2°C showed an intermediate mean V/C_{in} ratio (0.68).

Figure 13. The effect of temperature on the percentage of the filtered water which appears as urine in the white sucker. The data may be divided into three different groups according to the history of the fish before the experiment as follows: ● group three, fish Nos. 33 and 34 which were held in the laboratory for 10 days at 8°C; ○ group one, fish Nos. 3, 4, and 5 which were held for 30 days at 2°C; and ◐ group two, fish Nos. 6, 7, 8, and 9 which were held for 30 days at 4°C. V = urine flow and C_{in} = GFR.

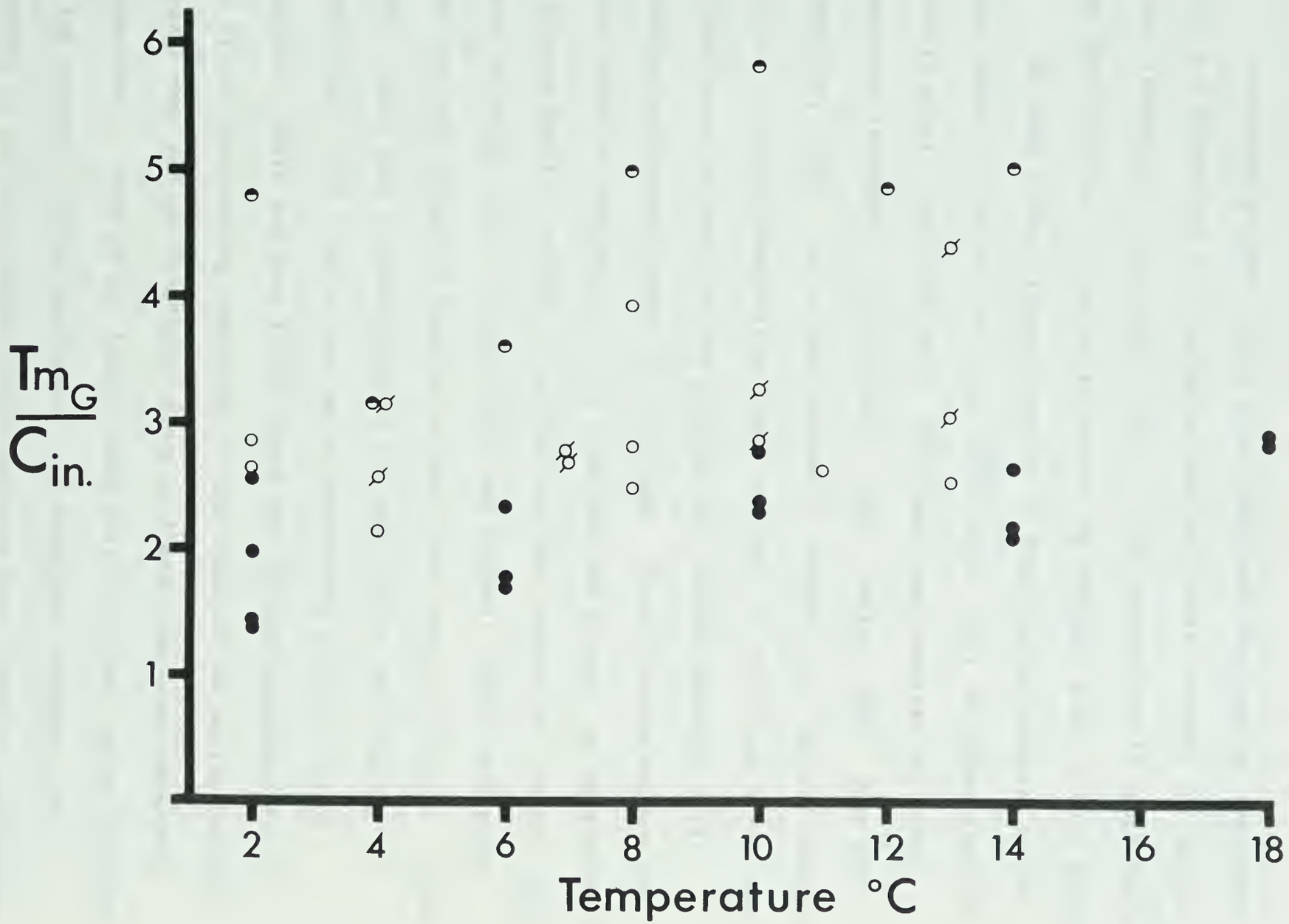

100
90
80
70
60
50
40
30
20
V/C in x 100
2
4
6
8
10
12
14
Temperature °C

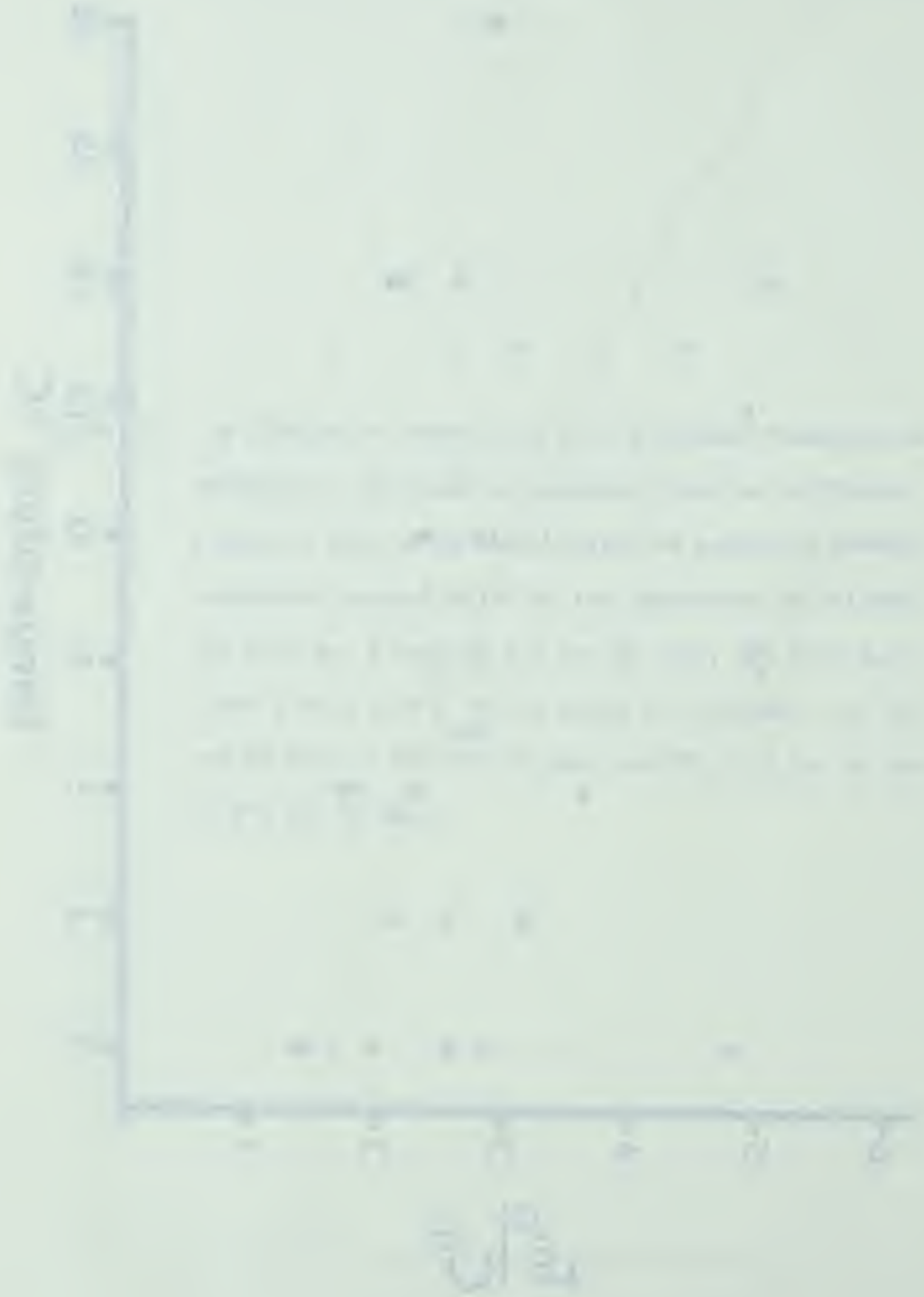
The Effect of Temperature on Tubular Glucose Reabsorption

The data which was used to determine the effects of temperature on the glucose reabsorptive mechanism was obtained from seven fish which were held in the laboratory for different periods of time at different temperatures. These fish can be divided into four groups according to their history prior to the experiment as follows: fish No. 3 was held for thirty days at 2°C; fish Nos. 6 and 7 were held for thirty days at 4°C; fish Nos. 12, 16, and 18 were held for thirty days at 8°C; and fish No. 34 was held for 10 days at 8°C.

Since a linear relationship exists between glucose transport maximum (Tm_G) and inulin clearance (C_{in}) (Fig. 10), the quantity of glucose reabsorbed per millilitre of filtrate formed will be a constant which is independent of filtration rate at a given temperature (Fig. 11). In order to evaluate the effect of temperature on the glucose transport mechanism itself, the ratio Tm_G/C_{in} was plotted against the temperature at which the determinations were made (Fig. 14. Appendix Table 10). The ratio for individual fish of Tm_G/C_{in} appears to increase slightly with increasing temperature. The average Q_{10} value obtained from eye-fitted lines through the four different sets of points was 1.11. The range of Q_{10} values was 1.0 for the fish held at 4°C for thirty days to 1.21 for the fish that was held at 8°C for ten days. Thus temperature has a very limited effect on the rate of tubular glucose transport. The quantity of glucose reabsorbed per millilitre of filtrate varied greatly, both within the groups and among the groups (Fig. 14). The fish which had been held at 2°C for thirty days showed a much higher reabsorptive capacity over the temperature range 2 to 14°C than did any of the other fish.

Figure 14. The effects of temperature on the glucose reabsorptive mechanism. The data was obtained from four different groups of fish which were classified according to their laboratory history prior to the experiment as follows: (θ) fish No. 3 held at 2°C for 30 days; (ø) fish Nos. 6 and 7 held at 4°C for 30 days; (0) fish Nos. 12, 16, and 18 held at 8°C for 30 days and (●) fish No. 34 held at 8°C for 10 days.


$\frac{Tm_G}{C_{in.}}$
6
5
4
3
2
1
2
4
6
8
10
12
14
16
18
Temperature °C



DISCUSSION

The plasma glucose levels which have been reported for freshwater fish are higher and more variable than the levels reported for marine forms (Kiermeir, 1939; Pavlov, 1939). This observation is supported by the data obtained for the white sucker and northern pike in this study.

The mean plasma glucose level of 66 female white suckers, which were caught in the trap net, was 129 ± 40 mg.%. This is higher than the levels, usually under 100 mg.%, which have been reported for other temperate freshwater fish (Kiermeir, 1939. Pavlov, 1939. Dean and Goodnight, 1964). The values reported by Kiermeir and Pavlov, however, are not strictly comparable to those obtained in this study since their methods were not specific for glucose, but instead measured total reducing substances present in the blood. The difference between the total reducing substances in the blood and the glucose, as determined by a glucose specific method, is called the "rest reduction" (White, 1928; Moule and Nace, 1963). White (1928) and Kiermeir (1939) have shown that the rest reduction level of teleost blood was significant. Falkmer (1961) found that glucose accounts for only 18% of the reducing substances in the blood of the marine short-horn sculpin, *Cottus scorpius*. Moule and Nace (1963) found that glucose accounted for 63 and 73% of the reducing substances in the blood of the marine toadfish, *Opsanus tau*, and the freshwater brown bullhead, *Ictalurus nebulosus*, respectively. Nace *et al.* (1964) found that in winter, values obtained by the enzymatic method approached, and sometimes exceeded, those obtained by the Folin method, which measures total reducing substances. They did not explain this apparent discrepancy. No reports of blood

sugar levels in the white sucker or other catostomids could be found in the literature. Kiermeir (1939) has reported values obtained by her and others for some of the cyprinids, which are closely related phylogenetically to the suckers and occupy similar habitats. The highest average blood sugar level she reported for a cyprinid was 100 mg.% for the tench, *Tinca vulgaris*. The discrepancy between plasma glucose levels obtained in this study and those measured by previous workers cannot be explained by the different methods used because the total reducing substances in the blood must be equal to or greater than the total glucose concentration. Methods which measure total reducing substances, however, are usually carried out on whole blood samples. The hemoglobin and plasma proteins are precipitated and the reducing power of the protein-free supernatant is then determined. Since the glucose determinations in this study were carried out in plasma, the differences between the two methods could be explained if the concentration of glucose inside the red cells was less than that in the plasma.

Following a post-spawning hyperglycemia of over 130 mg.% in early June, plasma glucose levels in male white suckers decreased steadily to less than 100 mg.% in September and October. No change of this sort could be detected in the data obtained for female fish. The decrease in plasma glucose levels observed in the male fish from July through October could be a secondary consequence of changes in the level of male sex hormones. Even during the most rapid periods of growth, the female gonad does not grow as rapidly as that of the male during July and August. The growth of both the male and female gonads requires the deposition of considerable quantities of energy reserves in the form

of both carbohydrate and fat. During the fall, the male gonad contains 2 1/2 times as much lipid as the female gonad (100.0 mg. lipid/gm. dry wt. as compared to 41.3 gm. lipid/gm. dry wt.) (D. Armstrong, unpublished data). Since this lipid is deposited in the male gonad over a very short period of time, the turnover of blood glucose to be used in fat synthesis must be very great during July and August. Hormonal stimulation of lipid synthesis and gonadal growth could result in lowering the plasma glucose levels. The female gonad develops more slowly throughout the year when water temperatures are above 2 or 3°C. Although it attains a much larger size than the male gonad, the female gonad never grows as rapidly as the male gonad does during July or August. Hence, the turnover of blood glucose used in gonadal growth is never as great in the female as in the male. Nace *et al.* (1964) found a seasonal variation in the blood glucose of the marine toadfish, *Opsanus tau*. Pavlov (1939) found that maturation of the gonad in four mature pike-perch, *Lucioperca luciop* was accompanied by an increase in blood sugar from a normal level of just over 100 mg.% to a mean level of 150 mg.%. She also reported hyperglycemia in certain salmonids immediately following spawning. It appears however that, in some cases at least, the spawn was removed by the experimenter immediately prior to blood sampling; hence, the hyperglycemia could, in part, be the result of handling. Thus it appears that the seasonal changes in blood sugar level which occur in different species of fish may in some species be related to the annual reproductive cycle.

The work of Dean and Goodnight (1964) inferred that temperature might be an important factor in controlling seasonal changes in the blood glucose level of some freshwater fish. They found that the blood

glucose levels in the black bullhead, *Ictalurus melas*, and the white crappie, *Pomoxis annularis*, increased from 48 and 91 mg.% respectively at 20°C to 62 and 180 mg.% respectively at 5°C. The blood glucose level of the blue-gill, *Lepomis macrochirus*, however, decreased from 88 mg.% at 20°C to 50 mg.% at 5°C. They found that temperature had no effect on the blood glucose level of the largemouth bass, *Micropterus salmoides*, which remained at 44 to 45 mg.% at both 5 and 20°C. Kiermeir (1939) was unable to detect any effect of temperature on the blood sugar level of the trout, *Trutta iridea (Salmo gairdneri)*. No direct evidence was obtained in the present study to indicate that temperature was important in controlling changes in plasma glucose levels of the white sucker or northern pike.

Due to difficulties in catching these fish, blood samples were not obtained from white suckers during every month of the year. The trap net could not be set during November, when the ice was forming on the lake, or in April, when the ice was melting. Although the trap net was set from early December until the end of March, a sufficient number of suckers was not caught for valid samples to be taken. Neither could suckers be caught in significant numbers in gill nets during January, February or March. This suggests that these fish were quite inactive when water temperatures fell below 2°C. Suckers may respond to low temperature and decreased photoperiod or low light intensity by decreased swimming and feeding activity. Such changes in activity could be similar to those described for the crucian carp, *Carassius carassius*, which apparently undergoes cold narcosis in the mud of pond bottoms under similar conditions (Roberts 1960). Roberts reported that shifts in metabolic pathways accompanied this narcosis. Such

shifts could result in altered plasma glucose levels.

The values obtained for the two male white suckers sampled in December indicates that the blood glucose levels in the male increase when water temperatures decrease. However, sufficient data is not available to be certain. The sexual cycle appears to be more closely associated with changes which occur in male sucker plasma glucose levels than is temperature.

The normal plasma glucose levels obtained for northern pike in this study exceeded those reported for the blood of pike by Gray and Hall (1929), who found 75 mg.% and by Kiermeir (1939), who found an average of 66 mg.% for three fish. Only the mean value of 142 mg.%, reported by McCay (1931) for eight pike, is comparable to the values obtained for the male pike sampled from the trap net in this study. None of the reported values approaches that found for female pike from the trap net during June and July. The differences in plasma glucose levels obtained for pike in this study and those values reported by other workers, might be due to seasonal changes in blood sugar levels Such a change could be due to temperature effects on blood sugar levels similar to those reported by Dean and Goodnight (1964) for other temperate freshwater fish. The effects of temperature on the blood sugar level of northern pike, apparently, have not been investigated. On the other hand, seasonal changes in plasma glucose levels could also be related to the sexual cycle. The high values obtained from the recently spawned fish, particularly females, sampled in this study indicates that a post-spawning hyperglycemia is present. Again the method of obtaining the fish and the sampling procedures used, can markedly effect the glucose level and in many cases this is

not reported in the literature.

Data from the white sucker indicates that significant differences exist in the plasma glucose levels between male and female fish in September and October. During these months the plasma glucose levels of the male fish were 37 mg.% lower than those found in female fish. Similarly, during June and July the plasma glucose level of male northern pike was 79 mg.% lower than the female level. This is a much greater difference than the 4 mg.% by which male toadfish exceeded females immediately prior to spawning (Nace *et al.*, 1964). Since northern pike were caught by trap net only during June and July, the duration of the sexual difference in plasma glucose levels could not be determined for this species.

A second sexual difference which was detected in both the northern pike and the white suckers was the significantly greater variation, as indicated by the standard deviation, in plasma glucose levels of female fish as compared to male fish from the same population. With the exception of the female white suckers, the standard deviation of the plasma glucose levels for northern pike and white suckers do not differ significantly from those reported by other workers for freshwater fish (Dean and Goodnight, 1964; Pavlov, 1939). Standard deviations of thirty per cent or greater were reported by Dean and Goodnight (1964) for the white crappie, *Pomoxis annularis*, and by Pavlov (1939) for the perch, *Perca fluviatilis*, and the gwyniad, *Coregonus lavaretus*.

The opportunity for fish to feed while in the trap net is limited, and the length of time that fish had been captured could vary from a few hours up to one week. Since the fish used for normal

plasma glucose levels in this study were caught by trap net, a variety of nutritional states was probably represented in each sample. Differences in the nutritional state of the fish included in a sample could increase the variability of the plasma glucose levels of that sample.

Perhaps the most difficult aspect of a study which purports to measure normal levels of any physiological parameter is to obtain samples from animals which are physiologically representative of that species under natural conditions. The plasma glucose levels of fish from the trap net in December was 136 ± 29 mg.% as compared to 186 ± 29 mg.% for fish caught by gill net during the same month. Presumably the difference is due to struggling by the fish when caught in the gill net. Previous workers have shown that strenuous exercise (Secondat, 1950; Hammond, 1964) can alter the blood glucose level of freshwater fish for considerable periods of time. Hammond (1964) found that plasma glucose levels of the rainbow trout, *Salmo gairdneri*, increased following strenuous exercise. The crib of the trap net used in this study was large enough to permit freedom of movement of the fish. The net was only partially lifted from the water when it was to be emptied so that the fish had room to swim freely until removed. Fish caught by gill net, however, struggled until they were exhausted or until they were removed from the net. Even during strenuous exercise, plasma glucose levels do not change within one minute of the initiation of exercise (Hammond, 1964). Since all blood samples were taken within one minute of the time that the fish was first handled, the sampling procedure would not be expected to affect the plasma glucose level.

The plasma glucose level of northern pike caught by gill net during the winter was $123^{\pm}56$ mg.% as compared to $131^{\pm}16$ mg.% for male fish and $210^{\pm}92$ mg.% for female fish caught by trap net in June and July. If struggling in the gill net has the same effect on pike plasma glucose levels as it appears to have on the plasma glucose level of the sucker, the normal plasma glucose levels of northern pike in the winter would be considerably lower than 123 mg.%. This would bring plasma glucose levels of northern pike in the winter down to the range which has been previously reported for these fish (Gray and Hall, 1929; Kiermeir, 1939; Pavlov, 1939). In any case, capture by gill net would not likely lower the plasma glucose level of a fish. Consequently, the relatively low plasma glucose levels of pike caught by gill net in the winter suggest that seasonal changes also occur in their plasma glucose levels.

The high, extremely variable, plasma glucose levels found in laboratory held fish is probably the result of handling and of the laboratory environment. Even after being held in the laboratory for a week, white suckers were very easily disturbed. A noise or sudden movement caused them to dart wildly about in their large holding tank or to struggle in the leucite boxes. These fish were constantly subject to intermittent noises and vibrations from pumps and automatic temperature controllers. Thus, sensitivity to external stimulii could be the cause of elevated plasma glucose levels.

The most striking feature of urine flow records for individual white suckers is the variation in flow rate that occurred from hour to hour. The urine flow during one hour would frequently be twice that or one-half that of the previous hour. Even when the average

urine flow of successive ten-hour intervals is compared in a single fish, the flow rate may double or be halved between any two successive intervals. At constant temperature, the average urine flow over ten-hour intervals, when calculated on a body weight basis, does not vary any more between individual fish than in a single fish during successive intervals. Such variation could be the result of changes in the glomerular filtration rate, or of changes in tubular reabsorption of water, or of some combination of both.

The rate of urine production in the white sucker, as in other lower vertebrates, appears to be primarily controlled by intermittent glomerular function. Evidence for this type of control is seen in the linear relationships which exist between water reabsorption and glomerular filtration rate, as well as glucose transport maximum and the glomerular filtration rate. The linear relationship between GFR and urine flow indicates that it is the rate of filtrate formation rather than the tubular permeability to water that is important in controlling the volume of urine produced. Filtration rate, in turn, is controlled by controlling the number of glomeruli functioning at one time, rather than by reducing the amount of filtrate formed by each glomerulus. If graded activity of all the glomeruli were responsible for the variations in filtration rate, the rate of water reabsorption would decrease as filtration rate increased, and the glucose transport maximum would be independent of filtration rate. The linear relationship between glomerular filtration rate and tubular reabsorption of glucose or water, however, indicates that graded glomerular activity is not the major glomerular parameter involved in the renal function of white suckers. Graded glomerular

activity, however, may account for the variations which have been observed in the relationship between GFR and urine flow and between GFR and Tm_G in individual fish.

When the variations in Tm_G arising from intermittent glomerular activity are eliminated by dividing Tm_G by GFR, a positive relationship was found to exist between the percentage water reabsorption and the quantity of glucose reabsorbed per millilitre of filtrate produced. The glucose is presumably actively reabsorbed in the proximal tubule, and the water is passively reabsorbed in the distal tubule. These two processes are not related and in fact occur in anatomically different portions of the tubule. The relationship between glucose reabsorption and the percentage water reabsorbed, then, can only be explained by variations in the rate of movement of the fluid column through the tubule. When the glucose reabsorptive mechanism is saturated, the quantity of glucose reabsorbed from one millilitre of glomerular filtrate will be a function of the length of time that the filtrate was in the proximal tubule. Similarly, the quantity of water reabsorbed in the distal tubule will be a function of the flow rate through the distal tubule. Variations in the rate of movement of the fluid column can be accomplished by graded filtration.

Hickman (1965) suggested that the concept of graded glomerular intermittancy could explain his observations concerning the regulation of urine flow in the white sucker. Glomerular intermittancy accounted for the linear relationship he observed between urine flow and GFR, while the variations in water reabsorption in individual fish were attributed to graded glomerular activity. The linear relationship between glucose reabsorption and glomerular filtration rate observed

in the present study confirm the importance of glomerular intermittency in controlling urine flow. The results of the present study also indicate that just over fifty per cent of the observed variation in water reabsorption in the white sucker can be accounted for by graded glomerular activity.

Striking differences occurred in water reabsorption among individuals which were caught at different times and among individuals which were caught at the same time but held under different conditions in the laboratory. All of the fish which were caught at the same time and treated in an identical fashion, however, showed similar percentages of water reabsorption. Hickman (1965) attributed the short term variation in water reabsorption within a single fish to graded glomerular activity rather than to hormonal involvement. This does not, however, explain the differences in water reabsorption which occurred between groups of fish caught at different times or between groups of fish caught at the same time but held under different conditions in the laboratory.

Differences in the relationship of Tm_G to GFR also occurred among individuals. The differences among individuals in both water reabsorption and in glucose reabsorption suggest that either some basic change takes place in both the proximal and distal tubule or that the rate of filtration by all of the glomeruli in the functioning nephron population changes. If the latter is true, fish with relatively higher Tm_G values should also show a greater percentage of water reabsorption; this is generally found to be the case.

The rate of urine flow in freshwater fish represents the net permeability of the fish to the water of its environment (Wikgren, 1953).

The Q_{10} value for urine flow in the sucker remained nearly constant with an average value of 2.2 over the temperature range 2 to 18°C. The increase in urine flow is due to a direct effect of temperature on the total permeability of the fish to water. It would be more accurate, therefore, to say that the permeability of the fish has a Q_{10} value of 2.2. Urine flow did not increase rapidly at low temperatures as Wikgren (1953) found in the lamprey or at temperatures between 15 and 20°C as Pora and Prekup (1960) found in the crucian carp, *Cyprinus carassius*. Wikgren (1953) found a Q_{10} for the permeability of the lamprey, *Petromyzon fluviatilis*, of 4.8 over the temperature range 9 to 18°C. Pora and Prekup (1960) found the highest Q_{10} values for urine flow in the crucian carp, between 15 and 20°C with a Q_{10} of 14.5 over that temperature range. They found that between 6 and 15°C the Q_{10} value for urine flow was 2.4 and between 20 and 28°C the Q_{10} value was 1.1.

All of the fish used to investigate the effects of temperature on urine flow were caught during late fall and early winter when environmental water temperatures were as low as 2°C. The urine flow of suckers at 2°C was found to be 1.25 ml./kg./hr.; this is nearly twice the mean value reported by Haywood and Clapp (1942) for white suckers of comparable size sampled in the winter. These investigators did not state the temperature at which their determinations were made, but presumably it was not below 2°C. The white suckers used for normal urine flow values in this study were caught during December and January, while those used by Haywood and Clapp (1942) were caught during January and February. The apparent seasonal variation in urine flow found by Haywood and Clapp (1942) could have been due to holding the fish at different temperatures during the determinations.

Water reabsorption in the kidney was not significantly affected by temperature over the range of 2 to 14°C. This is in sharp contrast to total body permeability to water which increased markedly with increasing temperature. Hickman (1965) indicated that no hormone had been reported to produce significant changes in tubular water reabsorption in freshwater fish. The major function of the kidney in freshwater fish is the elimination of water. High rates of tubular reabsorption of water reduce the efficiency of this process. If the permeability of the distal tubule increased with increasing temperature, as does the apparent premeability of the external epithelium, the rate of tubular water reabsorption would increase and the efficiency of water excretion would decrease. Since the permeability of the distal tubule is not apparently affected by temperature, the efficiency of water excretion would not be affected by increasing temperature.

Increasing temperature had a small effect on the renal glucose transport mechanism in the white sucker. Variations in the quantity of glucose reabsorbed per millilitre of filtrate formed, occurred at all temperatures investigated. Such variations could be explained by a graded glomerular activity over the entire filtration range. The lack of a significant temperature effect on the glucose transport mechanism itself is evidence that this mechanism is very stable.

Renal tubular processes in the white sucker have developed temperature independence as is reflected in the low Q_{10} values that the tubular process of water reabsorption and glucose transport show. Temperature significantly influences the apparent body permeability to water. The gills and associated structures comprise the major portion of the body surface exposed to the environment. At high

temperatures, the respiratory rate of fishes increases causing an increased flow of blood through the respiratory capillaries. This would result in an effective increase in the quantity of blood which is brought into contact with the environment, and a consequent increase in the quantity of water taken into the blood across the respiratory surfaces. Temperature may in fact have no direct effect on the actual permeability of the respiratory membranes to water.

SUMMARY

1. Plasma glucose levels were determined for white suckers sampled at intervals from April until December, and for northern pike during June and July, and from December until March. Plasma glucose levels of fish caught by both gill net and trap net were compared. Some plasma glucose values are also reported for fish held in the laboratory.

2. The plasma glucose level of male white suckers sampled by trap net decreased from 141 ± 32 mg.% in June, to 93 ± 11 mg.% in September and October. No change could be detected in the plasma glucose level of female fish. The average plasma glucose level of 66 female fish was 129 ± 40 mg.%.

3. When fish were caught by trap net, the standard deviation of plasma glucose levels for male fish of both species was approximately one-half that of the female fish. The average plasma glucose level of female white suckers in August, September and October (123 mg.%) was significantly higher than that of male fish (97 mg.%) during the same period. Female northern pike had significantly higher blood sugar levels (210 mg.%) than male pike (131 mg.%) during June and July.

4. White suckers which were caught by gill net (186 ± 87 mg.%) and those sampled in the laboratory (199 ± 87 mg.%) showed higher and more variable plasma glucose levels than fish which were sampled from the trap net. No sexual difference could be detected in the plasma glucose levels of fish sampled in the

laboratory or in those caught by gill net.

5. A linear relationship existed between urine flow and inulin clearance as well as between glucose transport maximum and inulin clearance, indicating that glomerular intermittency is the primary means by which the rate of urine production is controlled in the white sucker.

6. A correlation existed between the percentage of the filtered water and the proportion of the filtered glucose which was reabsorbed. Such a correlation indicates that graded glomerular filtration is also present in the white sucker, but its importance in controlling urine volume is secondary to intermittent glomerular filtration.

7. Urine flow increased with increasing temperature with a Q_{10} value of 2.2. Temperature did not significantly affect the percentage of filtered water which was reabsorbed or the rate of glucose reabsorption.

BIBLIOGRAPHY

Carl, G. C., Clemens, W.A., and Lindsey, C. C. 1959. *The Freshwater Fishes of British Columbia.* British Columbia Provincial Museum, Victoria, B. C. 192 pp.

Croxton, F. E. 1953. *Elementary Statistics with Applications in Medicine and the Biological Sciences.* Dover Publications Inc., New York. 376 pp.

Dantzler, W. H. and Schmidt-Nielsen, B. 1966. Excretion in freshwater turtle (*Pseudemys scripta*) and desert tortoise (*Gopherus agassizii*). *Am J. Physiol.* 210:198-209.

Dean, J. M. and Goodnight, C. J. 1964. A comparative study of carbohydrate metabolism in fish as affected by temperature and exercise. *Physiol. Zool. 37:*280-299.

Falkmer, S. 1961. Experimental diabetes research in fish. *Acta Endocrinol. 37*:Suppl. 59.

Forster, R. P. 1942. The nature of the glucose reabsorptive process in the frog renal tubule. Evidence for intermittency of glomerular function in the intact animal. *J. Cell. Comp. Physiol. 20:*55-69.

Forster, R. P. 1953. A comparative study of renal function in marine teleosts. *J. Cell. Comp. Physiol. 42*:487-509.

Gray, I. E. 1928. The effect of insulin on the blood sugar of fishes. *Am. J. Physiol. 84*:566-573.

Gray, I. E. and Hall, F. G. 1929. The distribution of sugar in the blood of fishes. *J. Elisha Mitchell Sci. Soc. 45:*142. Cited by Kiermeir, 1939.

Gray, I. E. and Hall, F. G. 1930. Blood sugar and activity in fishes with notes on the action of insulin. *Biol. Bull. 58:*217-223.

Hammond, B. R. 1964. The effect of physical conditioning on the metabolism of lactate, phosphate, and glucose in rainbow trout, *Salmo gairdnerii.* M. Sc. Thesis U. of Alberta, Edmonton.

Haywood, C. and Clapp, M. J. 1942. A note on the freezing-points of the urines of two fresh-water fishes: the catfish (*Ameiurus nebulosus*) and the sucker (*Catostomus commersonii*). *Biol. Bull. 83*:363-366.

Hickman, C. P. Jr. 1965. Studies on renal function in freshwater teleost fish. *Trans. R. Soc. Can. 3:*(4) 213-236.

Higashimura, T., Yamada, T., Nohara, N., and Shidei, T. 1962. External standard method for the determination of the efficiency in liquid scintillation counting. *Int. J. App. Radiation and Isotopes. 13*:308.

Keston, A. S. 1956. Specific colorimetric enzymatic reagents for glucose. *Abstracts of Papers, 129th meeting, A.C.S.* p. 31c.

Kiermeir, A. 1939. *Über Den Blutzucker Der Süsswasserfische.* Z. Vergl. Physiol. 27:460-491.

Lahlou, B. 1966. Eise en évidence d'un "recrutement glomérulaire" dans le rein des Téléostéens d'après la mesure du Tm glucose. *C. R. Acad. Sci. Paris. 262:*1356-1358.

McCay, C. M. 1931. Phosphorus distribution, sugar and hemoglobin in the blood of fish, eels, and turtles. *J. of Biol. Schem. 90:*497-505.

McCormick, N. A. and MacLeod, J. J. R. 1925. The effect on the blood sugar of fish of various conditions including the removal of the principal islets (isletectomy). *Proc. Roy. Soc. London. Ser. B. 98:*1-29.

Moule, M. L. and Nace, P. F. 1963. Reducing substances in the blood of normal and alloxan-treated fish. *Can. J. Biochem. Physiol. 41:*2397-2407.

Nace, P. F., Moule, M. L., and Schuh, J. E. 1964. The normal blood sugar of the toadfish. *Can. J. Physiol. and Pharm. 42:*225-232.

Pavlov, V. A. 1939. Hemoglobin and sugar content in the blood of some freshwater fishes (a comparative study). *Bull. Inst. Freshwater Fisheries (Russia). 21:*120-142.

Pitts, R.F. 1963. *Physiology of the Kidney and Body Fluids.* Year Book Medical Publishers Inc. Chicago. 243 pp.

Pora, A. W. and Prekup, O. 1960. (Translation) A study of excretory processes of freshwater fish. Part II. Influence of environmetal temperature on the excretory processes of the carp and Crucian carp. *Voprosy Ikhtiol. 15:*138-147.

Richards, A. N., Westfall, B.B., and Bott, P. A. 1934. Renal excretion of inulin, creatinine and xylose in normal dogs. *Proc. Soc. Exper. Biol. and Med. 32:*73.

Roberts, J. L. 1960. The influence of photoperiod upon thermal acclimation by the Crucian carp, *Carassius carassius. Verh. Deutsh. Zool. Gesellsch. Bonn.* pp. 73-78.

Saifer, A. T. and Gerstenfeld, S. 1958. The photometric microdetermination of blood glucose with glucose oxidase. *J. Lab. and Clin. Med. 51:*448-459.

Schmidt-Nielsen, B. and Forster, R. P. 1954. The effect of dehydration and low temperature on renal function in the bullfrog. *J. Cell. Comp. Physiol. 44:*233-241.

Secondat, M. 1950. Influence de l'exercise masculaire sur la valeur de la glycemie de la Carpe *(Cyprinus carpio). C. R. Acad. Sci. Paris. 231:*796-797.

Shannon, J. A. and Smith, H. W. 1935. The excretion of inulin, xylose, and urea by normal and phlorinized man. *J. Clin. Invest. 14:*393.

Simpson, W. W. 1926. The effects of asphyxia and isletectomy on the blood sugar of *Myoxocephalus* and *Ameiurus. Am. J. Physiol. 77:*409-418.

Smith, H. W. 1956. *Principles of Renal Physiology.* Oxford University Press. New York. 236 pp.

Tanner, G. A. and Klose, R. M. 1966. Micropuncture study of inulin reabsorption in Necturus kidney. *Am. J. Physiol. 212:*1036-1038.

Teller, J. D. 1956. Direct quantitative, colorimetric determination of serum or plasma glucose. *Abstracts of Paper, 130th meeting, A. C. S.* p. 69c.

Wikgren, B. 1953. Osmotic regulation in some aquatic animals with special reference to the influence of temperature. *Acta Zoologica Fennica. 71:*1-102.

White, F. D. 1928. Reducing substances in the blood of the dogfish, *Squalus sucklii,* and certain other fishes. *J. Biol. Chem. 77:*655-669.

APPENDIX

Table 3. Three Metre Water Temperature in Lac Ste. Anne during 1966.

Date	Temp. C	Date	Temp. C	Date	Temp. C
May 1	4.0	July 9	19.4	Sept. 19	15.5
May 11	8.5	July 15	19.7	Sept. 23	15.2
May 19	9.0	July 22	18.2	Sept. 30	13.0
May 25	12.3	July 29	18.0	Oct. 7	12.0
June 2	12.3	Aug. 12	16.9	Oct. 14	7.8
June 9	14.2	Aug. 18	17.0	Oct. 21	5.0
June 13	15.0	Aug. 24	17.5	Oct. 29	4.2
June 18	17.5	Sept. 1	16.1	Dec. 19	1.5
June 28	18.2	Sept. 9	15.8	Dec. 22	1.4

Table 4. Seasonal changes in the gonadosomatic index (GSI) of male white suckers.

Date of Sample	n	Average GSI			Date of Sample	n	Average GSI		
		$\frac{G.W.}{B.W.}$	$2 \times \sigma_{\bar{x}}$	$\frac{G.W.}{F.L.}$			$\frac{G.W.}{B.W.}$	$2 \times \sigma_{\bar{x}}$	$\frac{G.W.}{F.L.}$
July 9	4	0.81	0.06	0.19	Dec. 20	6	5.89	0.49	1.50
Aug. 18	6	6.66	1.29	1.61	Jan. 16	3	5.92	-	1.43
Sept. 1	5	9.00	0.59	2.35	March 13	3	6.32	-	1.57
Sept. 9	5	8.45	1.27	2.12	April 21	3	6.36	0.74	1.56
Oct. 4	6	7.29	0.87	1.98	May 26	3	2.71	0.84	0.70
Oct. 20	9	6.89	1.83	1.64	June 9	2	0.59	-	0.50
Dec. 15	5	6.31	0.71	1.55	June 18	5	1.93	0.61	0.43

Symbols used: n = number of individuals in the sample, G.W. = gonad weight, B.W. = total body weight, F.L. = fork length, $\sigma_{\bar{x}}$ = one standard error of the mean G.W./B.W. ratio.

Table 5. Seasonal changes in the gonadosomatic index (GSI) of female white suckers.

Date of sample	n	Ave. GSI	2x $\sigma_{\bar{x}}$	Date of sample	n	Ave. GSI	2x $\sigma_{\bar{x}}$
July 7	3	2.25	0.26	Dec. 15	7	11.63	1.61
July 15	5	1.98	0.80	Dec. 21	13	12.23	0.93
July 22	12	3.06	0.36	Jan. 16	3	11.88	-
Aug. 11	6	4.08	0.37	Feb. 19	3	11.79	-
Aug. 18	11	5.26	0.68	March 13	3	12.00	-
Sept. 1	5	6.11	0.82	March 23	8	13.61	-
Sept. 9	11	7.02	0.70	April 21	9	15.58	0.75
Oct. 7	10	8.85	0.84	May 26	6	17.67	1.97
Oct. 11	10	9.20	0.71	June 2	5	1.68	0.29
Nov. 7	3	9.06	1.50	June 18	4	2.12	0.53

Symbols used: n = number of individuals in the sample, Ave. GSI = average gonadosomatic index of the sample, $\sigma_{\bar{x}}$ = one standard error of the mean.

Table 6. Average plasma glucose levels of white suckers caught by trap net during 1966.

Date of sample	male fish				female fish			
	n	$\bar{x}$	σ_x	$\sigma_{\bar{x}}$	n	$\bar{x}$	σ_x	$\sigma_{\bar{x}}$
April 21	2	124	8.5	6.0	8	123	39.0	13.7
June 4	3	153	50.1	28.6	6	113	35.8	14.6
June 18	6	135	24.9	10.2	4	176	63.5	31.8
July 16	3	117	12.0	6.9	10	139	53.9	17.0
Aug. 11	-	-	-	-	6	112	28.4	11.6
Aug. 18	6	101	20.5	8.4	11	120	32.3	9.7
Sept. 9	3	96	14.1	8.2	6	146	42.3	17.2
Oct. 7	3	91	10.1	5.8	8	118	50.5	17.8
Dec. 22	2	139	40.3	28.5	8	135	28.8	10.2
Ave.			22.5	12.8		129	41.6	15.9

Symbols used: n = number of fish sampled, $\bar{x}$ = average plasma glucose level of sample, σ_x = one standard deviation of the plasma glucose level of the sample, $\sigma_{\bar{x}}$ = one standard error of the mean.

Table 7. Relationship between filtration rate (C_{in}), glucose transport maximum (Tm_G) and percent water reabsorption in the white sucker.

Fish #	Sample #	C_{in} (ml/hr)	Tm_G (mg/hr)	Tm_G/C_{in} (mg/ml)	% water reab.
39	1	2.77	10.62	3.83	55.1
"	2	2.94	11.40	3.78	35.1
"	3	3.17	13.00	4.10	47.7
39	4	3.32	11.92	3.60	39.9
"	5	3.78	14.59	3.86	42.4
"	6	4.31	19.27	4.47	35.3
40	1	0.75	2.92	3.89	55.8
"	2	2.50	17.35	6.93	45.4
"	3	1.91	13.74	7.18	66.9
40	4	3.16	21.98	6.96	49.7
"	5	2.38	7.44	3.13	32.9
"	6	0.21	1.03	4.81	48.1
18	9	2.80	8.06	2.88	53.4
16	6	2.86	6.15	2.15	9.9
12	5	2.16	5.73	2.65	29.3
7	2	3.53	11.17	3.17	35.4
6	2	1.98	5.12	2.59	44.0
3	1	2.84	12.40	4.37	44.2
3	4	5.31	16.77	3.16	32.3
"	7	5.89	21.29	3.62	33.9
34	2	1.27	2.32	1.82	17.0
34	3	0.28	0.50	1.77	20.5
"	4	1.17	2.91	2.49	43.6
"	6	0.93	2.97	3.19	44.6
34	7	1.25	2.78	2.23	18.1
"	8	0.80	1.73	2.16	11.7
"	9	0.66	1.94	2.93	29.7

Table 8. Effect of temperature on urine flow in the white sucker.

Temp. C	n	V	σ_x	$\sigma_{\bar{x}}$
2	7	1.21	0.49	0.19
4	8	1.51	0.28	0.10
6	12	1.83	0.71	0.20
8	6	1.86	0.26	0.11
10	11	2.45	0.81	0.24
12	2	3.71	0.30	0.21
14	4	3.12	0.94	0.47
18	2	4.39	0.63	0.45

Symbols used: n = the number of independent collection intervals, each consisting of at least 9 consecutive hours from a single fish; V = average urine flow for all the intervals (ml/kg/hr); σ_x = one standard deviation $\sigma_{\bar{x}}$ = one standard error of the mean.

Table 9. The effect of temperature on the percentage of water reabsorption.

T°C	F #	S #	% R	T°C	F #	S #	% R	T°C	F #	S #	% R
2	3	1	41.9	6	34	9	27.7	10	33	12	15.2
"	"	2	32.7	"	33	8	49.4	12	3	15	50.1
"	4	1	37.7	"	"	9	17.4	"	"	16	45.4
2	5	1	30.1	7	6	4	32.6	12	4	15	29.4
"	"	2	38.7	"	7	"	33.9	"	"	16	29.2
"	7	10	60.5	"	"	5	48.0	"	5	15	29.8
2	7	11	68.1	7	8	4	18.6	12	5	16	38.2
"	9	1	66.3	"	"	5	93.7	13	6	8	57.0
"	"	2	76.1	8	3	10	30.5	"	7	8	61.2
2	34	2	17.0	8	3	11	32.9	13	7	9	52.8
"	"	3	20.5	"	4	10	33.6	"	9	5	6.9
"	"	4	43.6	"	$	11	32.6	"	"	6	38.1
2	34	6	44.6	8	5	10	31.5	13	7	14	77.1
"	33	2	4.3	"	"	11	29.1	"	"	15	58.1
"	"	6	4.9	"	7	12	26.0	14	3	19	49.7
4	3	4	32.6	8	7	13	73.5	14	3	20	41.8
"	"	5	25.7	"	9	3	56.1	"	4	19	27.0
"	4	4	32.1	10	3	13	33.5	"	"	20	29.3
4	4	5	17.0	10	3	14	36.8	14	5	19	35.8
"	5	4	21.3	"	4	13	30.2	"	34	13	17.6
"	"	5	25.1	"	"	14	28.3	"	"	14	17.9
4	6	2	44.6	10	5	13	31.1	14	34	15	22.6
"	"	3	34.4	"	"	14	35.0	"	33	13	0.7
"	7	2	36.8	"	6	6	64.1	"	33	14	-2.2
4	7	3	39.5	10	6	7	44.2	14	33	15	-0.7
6	3	7	35.8	"	7	6	49.5	18	34	16	26.7
"	"	8	32.4	"	"	7	59.1	"	"	17	23.3
6	4	7	23.0	10	34	10	21.3	18	33	16	32.2
"	5	"	32.5	"	"	11	28.6	"	"	17	22.4
"	"	8	33.2	"	"	12	35.8				
6	34	7	18.2	10	33	10	13.2				
"	"	8	17.2	"	"	11	5.9				

Symbols used: T°C = Temperature at which sample was taken, F # fish number, S # = sample number from that fish, % R = percentage water reabsorption for that sample.

Table 10. The effect of temperature on the glucose reabsorptive mechanism.

Temp. C	Fish #	Sam. #	$\frac{Tm_G}{C_{in}}$	Temp. C	Fish #	Sam. #	$\frac{Tm_G}{C_{in}}$
2	34	2	1.46	12	3	15	4.87
"	"	3	1.40	14	"	19	4.90
"	"	4	1.99	4	6	2	2.59
2	34	6	2.55	4	7	2	3.17
6	"	7	1.78	7	6	4	2.76
"	"	8	1.72	"	7	4	2.72
6	34	9	2.34	10	6	6	2.83
10	"	10	2.38	"	7	6	3.25
"	"	11	2.76	13	6	9	4.38
10	34	12	2.33	13	7	8	3.05
14	"	13	2.18	2	12	5	2.65
"	"	14	2.12	"	16	9	6.45
14	34	15	2.64	2	18	9	2.87
18	"	16	2.81	4	16	6	2.15
"	"	17	2.89	8	12	2	2.84
2	3	1	4.80	8	16	2	2.49
4	"	4	3.16	"	18	11	3.92
6	"	7	3.61	11	"	12	2.66
8	3	10	4.99	13	12	7	2.52
10	"	13	5.80				

Symbols used: Tm_G = glucose transport maximum (mg/hr), C_{in} = inulin clearance (ml/hr).